

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 April 2001 (26.04.2001)

PCT

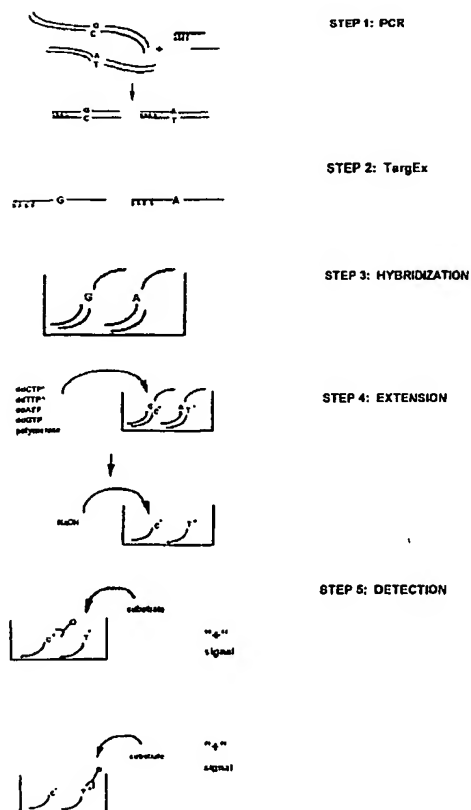
(10) International Publication Number
WO 01/29262 A2

- (51) International Patent Classification⁷: **C12Q 1/68**
- (21) International Application Number: **PCT/US00/28436**
- (22) International Filing Date: **13 October 2000 (13.10.2000)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
60/160,096 **15 October 1999 (15.10.1999)** **US**
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US **60/160,096 (CIP)**
Filed on **15 October 1999 (15.10.1999)**
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- (81) Designated States (national): **AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL,**

[Continued on next page]

(54) Title: **GENOTYPING REAGENTS, KITS AND METHODS OF USE THEREOF**

Diagram of GBA Process



(57) Abstract: The present invention provides oligonucleotides that can be used to determine the presence, absence or identity of a single nucleotide polymorphism (SNP), kits containing such oligonucleotides, and methods of genotyping a nucleic acid sample using such oligonucleotides.

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IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

GENOTYPING REAGENTS, KITS AND METHODS OF USE THEREOF

Background

Sequencing the human genome has provided information to dramatically change the way diseases are studied, diagnosed and treated. The fields of pharmacogenomics and pharmacogenetics focus on the variability and diversity of DNA, and how this diversity can impact biology, including response to drugs. The most common form of genetic diversity is thought to come in the form of individual DNA bases being different than the corresponding base in the average population. These single nucleotide polymorphisms ("SNPs") are a natural form of mutation, which are hereditary in nature. SNPs are viewed as both direct and indirect markers for many human diseases. Therefore, the analysis, or "scoring," of SNPs to determine their role in disease and drug efficacy can lead to the development of powerful diagnostics and therapeutics.

Adverse drug reactions (ADRs) represent the leading cause of hospitalization and mortality in the U.S., resulting in over 1.5 million hospitalizations and 100,000 deaths per year (J. Lazarou, et.al. in *The Journal of the American Medical Association (JAMA)*, (1998, 1200-1205). In addition, many promising drugs developed by pharmaceutical companies never make it through clinical development because of genetically based adverse reactions (ADRs) in a small number of patients. Therefore, scoring SNPs can identify at risk patients before the drug is prescribed and salvage potentially

useful new drugs. Alternatively, patients with SNP-induced variations in their conditions, evident in diseases like hypertension, can be identified and then prescribed the drug most appropriate for their genetic variant. Therefore, SNP analysis will be used to streamline drug discovery and development leading to safer and more effective tailor-made drugs.

Accordingly, new reagents are needed that can be used to identify whether heretofore unknown SNPs are present or absent from a given nucleic acid sample. The present invention satisfies this need and provides related advantages as well.

Summary of the Invention

In accordance with the present invention, there are provided oligonucleotides that can be used to determine the presence, absence or identity of a single nucleotide polymorphism. Invention oligonucleotides include genotyping oligonucleotides that hybridize with a portion a nucleic acid sequence listed in column 4 of Table 1, or complementary sequence thereof, and amplification oligonucleotides.

In accordance with another embodiment of the invention, there are provided kits useful for determining the presence, absence or identity of a single nucleotide polymorphism. In one embodiment, an invention kit comprises an oligonucleotide that hybridizes with a portion of a nucleic acid sequence listed in column 4 of Table 1, or complementary sequence thereof. In another embodiment, an invention kit comprises amplification oligonucleotides of columns 1 and 2 of Table 1. Also provided is an invention kit comprising an

oligonucleotide that hybridizes with a portion of a nucleic acid sequence listed in column 4 of Table 1, or complementary sequence thereof and two amplification primers.

5 Another embodiment of the invention provides methods of genotyping a nucleic acid sample by hybridizing a genotyping oligonucleotide to a nucleic acid sequence listed in column 4 of Table 1, or complementary sequences thereof. The genotyping
10 oligonucleotide can then be employed in a variety of reactions, such as, for example, a primer extension reaction. Also provided is a method of genotyping a nucleic acid sample by amplifying a target nucleic acid sequence and performing a single-nucleotide primer
15 extension reaction employing a genotyping oligonucleotide that hybridizes to the target nucleic acid sequence.

Brief Description Of The Figures

Figure 1 shows a schematic for genotyping a heterozygous nucleic acid sample containing two target
20 nucleic acids using the particular primer extension reaction referred to as Single Nucleotide Primer Extension (SNPE) and/or GBA. In step 1, genomic DNA is amplified using a pair of PCR primers from columns 1 and 2 of the same row of Table 1 herein, in which the five
25 most 5' nucleotides of one of the two PCR primers are linked by four exonuclease-resistant phosphorothioated linkages (e.g., 5' C-(p)-G-(p)-C-(p)-A-(p)-GTCTCAGGCCAGCT 3', for SEQ ID NO:2, where "-(p)-" represents a phosphorothioate linkage). The PCR primers are
30 complementary to sequences flanking either side of a specific nucleotide site (e.g., the site of a SNP) in the genomic DNA, thus resulting in an amplified nucleic acid

(the target nucleic acid) containing this specific site. In step 2, the amplified PCR product target nucleic acid is subjected to T7 gene 6 exonuclease digestion, which removes the strand of the target nucleic acid that did not include the 5' phosphorothioated linkages, resulting in a single-stranded target nucleic acid. In step 3, the genotyping primer (or SNPE primer) is hybridized with the single stranded target nucleic acid such that the specific nucleotide of the target nucleic acid that is to be analyzed (i.e., the SNP) is not hybridized, and is immediately adjacent the 3' end of the genotyping primer. In step 4, a terminator reagent mixture comprising dideoxy terminators corresponding to A, C, G and T, together with a polymerase, are added to the target nucleic acid-genotyping primer complex. The polymerase extends the genotyping primer by a single base, consisting of one of the four terminators, where the added terminator base is complementary to the SNP to be determined. The terminator base that is added to the genotyping primer is labeled with a detectable marker. In the second part of step four, NaOH is added to dissociate the target nucleic acid strand which is washed away, while the genotyping primer, fixed to a solid support, remains. In step 5, a detection reagent is added, if necessary, to generate signal from the detectable marker. This can be performed in an ELISA format where an enzyme-linked antibody specific for the detectable marker of a terminator is contacted with the extended genotyping primer. When detectable markers that can be distinguished from each other are used, multiple alleles can be identified in a single well. For example, as illustrated in step 5, two alleles can be identified (C and T) by the sequential use of antibodies selective for each of the terminators (ddCTP and ddTTP).

Figures 2A and 2B show schematics for genotyping a homozygous nucleic acid sample using the Single Nucleotide Primer Extension (SNPE) by the enzyme-linked immunosorbant assay (ELISA) detection method after amplification using a phosphorothioated primer and exonuclease digestion. In figure 2A, the template strand indicated in the figure is a representative target nucleic acid molecule (such as those listed in SEQ ID NO:4n+4). The SNPE primer indicated in the figure is a representative genotyping primer (such as those listed in SEQ ID NO:4n+3). The nucleotide to be interrogated is indicated as the SNP to be typed. Primer extension is performed as described herein, in which the polymerase extension step can be performed in the presence of the terminators ddATP, ddGTP, fluorescein-ddCTP and biotin-ddUTP. The last two terminators are linked to detectable markers. Figure 2B shows the detection step after the single nucleotide primer extension results in the addition of fluorescein-ddCTP onto the 3'-end of the SNPE primer. The diagram illustrates the interaction of alkaline phosphatase-conjugated anti-fluorescein antibody and the generation of signal at OD₄₀₅ after the addition of PNPP (p-nitrophenyl phosphate) substrate. Since the biotin-ddUTP is not incorporated, the anti-biotin horse radish peroxidase (HRP) does not bind to the primer, no reaction with the TMB (tetramethylbenzidine) substrate occurs and, hence, no signal is generated at OD₆₂₀.

Figure 3 shows a scatterplot of OD₄₀₅ (X-axis) versus OD₆₂₀ (Y-axis) resulting from SNPE that demonstrates a result that is heterozygous. The PCR primers used were oligonucleotides of SEQ ID NOs:25 and 26. The SNPE primer used was an oligonucleotide with SEQ ID NO:27. The targeted nucleic acid with the suspected polymorphism contains the sequence of SEQ ID NO:28. SNPE

was performed using fluorescein-ddCTP and biotin-ddUTP. The Y-axis corresponds to signal obtained from ddUTP incorporation and the X-axis corresponds to signal obtained from ddCTP incorporation. Each circle (●) indicates a result with a specific test sample (unknown) from an individual genomic DNA. Plus (+) symbols are positive controls using a synthetic template. Crosses (x) are negative controls where no PCR is performed.

10 Detailed Description of the Invention

In accordance with the present invention, there are provided oligonucleotides that can be used to determine (genotype) the presence, absence or identity of a single nucleotide polymorphism (SNP) at a preselected site in a human genomic nucleic acid sample. The location of the SNP in each SNP-containing oligonucleotide set forth in column 4 of Table 1 (also referred to herein as SEQ ID NO:4n+4, wherein n=0 through 934) herein corresponds to the nucleotide position on each SEQ ID NO:4n+4 that is immediately adjacent the nucleotide complementary to the 3' end of the corresponding genotyping oligonucleotide set forth in column 3 of the same row of Table 1. The nucleotide set forth on SEQ ID NO:4n+4 corresponding to the SNP site corresponds to the predominant polymorphism.

As set forth in Table 1, each row of oligonucleotides disclosed corresponds to a set of oligos useful for genotyping a nucleic acid sample for the presence or absence, or identity of a particular SNP. The oligonucleotides set forth in columns 1 and 2 of Table 1 (also referred to herein as SEQ ID NO:4n+1 and SEQ ID NO:4n+2, respectively, where n=0 through 934) are

used as primers to amplify the nucleic acid sample having the corresponding SNP-containing oligonucleotide from the same row in Table 1 (referred to as SEQ ID NO:4n+4, where n=0 through 934). See Figure 1. Once the nucleic acid sample having the corresponding SNP-containing oligonucleotide has been amplified, the corresponding genotyping oligonucleotide from column 3 (referred to as SEQ ID NO:4n+3, where n=0 through 934) of the same row in Table 1 is used to genotype the target nucleic acid, preferably by a primer extension reaction.

As used herein, the term "oligonucleotide", "oligo" or grammatical variations thereof, refers to polynucleic acid that can be either DNA or RNA. Invention oligos can be produced by methods well-known in art such as described in the Examples hereinafter; or the oligos can be purchased from commercial oligonucleotide production companies.

As used herein, the phrase "SEQ ID NO:4n+3, wherein n=0 through 934" refers to the group of oligonucleotides in the Sequence Listing that are set forth in column 3 of Table 1 herein (where n=(row number - 1). For example, n=0 corresponds to SEQ ID NO:3, n=1 corresponds to SEQ ID NO:7, ..., n=934 corresponds to SEQ ID NO:3739 of the Sequence Listing. Likewise, SEQ ID NO:4n+1, SEQ ID NO:4n+2, and SEQ ID NO:4n+4, wherein n=0 through 934, refers to the groups of oligonucleotides set forth in columns 1, 2 and 4, respectively, of Table 1 herein.

The oligonucleotides set forth in column 3 of Table 1 (SEQ ID NO:4n+3, where n=0 through 934) are also referred to herein as genotyping oligonucleotides. In addition, for each SNP located in each oligonucleotide

set forth in column 4 of Table, there are at least four potential genotyping oligonucleotides (e.g., genotyping primers) that are hybridizable to the region one nucleotide removed from either 5' or 3' end of the SNP nucleotide (two for each strand). Two of the genotyping primers (one for each target nucleic acid strand) are complementary to the target nucleic acid and have their 3' ends one nucleotide 3' to the SNP nucleotide. These primers can be used, for example, in a polymerase primer extension reaction such as described in Goelet, P. et al., U.S. Pat No.5,888,819 and Nikiforov, T.T. et al., U.S. Pat. No. 5,518,900, which are herein incorporated by reference. The other two primers are complementary to the target nucleic acid and have their 5' ends one nucleotide 5' to the SNP nucleotide. These primers can be used in, for example, a ligase/polymerase genotyping reaction such as described in Nikiforov, T.T. et al., U.S. Pat. No. 5,679,524, or a nucleotide and terminator mixture reaction such as described in Soderlund et al., U.S. Pat. No. 6,013,431 or by Koster et al., U.S. Pat. No. 6,043,031, which is herein incorporated by reference.

In addition, the oligonucleotides set forth in column 3 of Table 1 (SEQ ID NO:4n+3, where n=0 through 934) can be made one nucleotide longer such that the nucleotide at the 3' end lies complementary to the SNP nucleotide. These primers can be used in alternate genotyping methods, for example, in allele-specific polymerase chain reaction (AS-PCR) (Newton et al., 1989, Nucl Acids Res 17:2503-2516), ligase chain reaction (LCR) (Barrany, 1991, PNAS, USA, 88: 189-193), or oligonucleotide ligation assay (OLA) (Landegren et al., 1988, Science 241:1077), where the 3' nucleotide of the genotyping primer determines the presence of the SNP nucleotide.

In addition, the oligonucleotides set forth in column 3 of Table 1 (SEQ ID NO:4n+3, where n=0 through 934) can be made one or more nucleotides shorter such that the genotyping primers are complementary to the target nucleic acid and have their 3' ends two or more nucleotides 3' to the SNP nucleotide. These primers can be used in, for example, a labeled nucleotide and terminator mixture reaction such as described in Soderlund et al., U.S. Pat. No. 6,013,431 or by Koster et al., U.S. Pat. No. 6,043,031, which is herein incorporated by reference.

The genotyping primer can be an oligodeoxyribonucleotide (DNA), an oligoribonucleotide (RNA), or a copolymer of deoxyribonucleotides and ribonucleotides. The genotyping primer can be either natural or synthetic. The genotyping primer can be synthesized either enzymatically in vivo, enzymatically in vitro, or non-enzymatically in vitro. The genotyping primer can include modifications of the ribose-phosphate backbone, such as phosphorothioate or methylphosphonate or other backbone modifications such as peptide nucleic acid (PNA). The genotyping primer can also include modifications of the nucleotide bases. The genotyping primer can be labeled with a detectable marker; this detectable marker can be different from any detectable marker present in the reaction mixture or any detectable marker attached to the target nucleic acid. In addition, for use with single-nucleotide primer extension reactions, the genotyping primer must be capable of hybridizing or annealing with nucleotides present in the target nucleic acid, immediately adjacent to, and upstream of, the nucleotide base to be identified. One way to accomplish the desired hybridization is to have the template-dependent primer be substantially

complementary or fully complementary to the known base sequence immediately adjacent to the base to be identified. The genotyping primer may be bound to a solid surface such as described in U.S. Pat. No. 5 5,610,287, incorporated herein by reference in its entirety. Multiple different genotyping primers can be immobilized on a surface to create an array, thus allowing the determination of multiple genotypes in a single reaction. Multiple different genotyping primers 10 with distinct attachment means ("tags") can be used for multiplex single nucleotide primer extension in solution and subsequently separated for discrete detection, such as on a nucleic acid array comprising nucleic acids complementary to the attachment means of the genotyping 15 primers ("tag arrays").

Genotyping oligonucleotides of the invention can be as long as the nucleotide distance between an amplification primer and the SNP nucleotide, preferably not overlapping the amplification primer sequence, 20 preferably less than 90 nucleotides, preferably less than 50 nucleotides, more preferably between 14 and 50 nucleotides, most preferably between 17 and 30 nucleotides.

When invention genotyping oligonucleotides are 25 used in, for example, single-primer-extension reactions, hairpin loop structures can form due to self-complementarity of the nucleotide sequences of the oligo/primer. In order to disrupt the hairpin loop structure formation, "single-nucleotide-spacers" can be 30 inserted in the midst of the hairpin loop structure. As used herein, the term "single-nucleotide-spacer" refers to any non-Watson-Crick moiety incorporated within an oligonucleotide for the purpose of weakening nucleic acid

duplex stability without altering the fidelity of the surrounding Watson-Crick base pairs. Thus, modifications to genotyping oligonucleotides contemplated herein are for the purpose of destabilizing duplex formation, specifically disfavoring intrastrand folding in favor of interstrand pairing. The location of single-nucleotide-spacers are set forth in the Sequence Listing and column 3 of Table 1 as an "X". Such moieties are well-known and available in the art and can include anucleosidic moieties, abasic moieties, non-naturally occurring nucleotide analogs, and non-Watson/Crick base moieties.

As used herein, the term "anucleosidic moieties" refers to moieties that approximate the spacing of the phosphoribose linkage. Thus, anucleosidic sites are contemplated herein as space-holders between neighboring phosphates along the oligo backbone. Examples of anucleosidic moieties for use in invention oligonucleotides include phosphoramidite-coupled multicarbon aliphatic chains that lack a nucleotide base and approximate the internucleotide spacing. For example, a C-3 linker used in DNA synthesis (3-(4,4'-Dimethoxytrityloxy)propyl-1-phosphoramidite) (Glen Research, Sterling, VA) will have the approximate spacing of one nucleotide within a nucleic acid chain. Other anucleosidic sites include ethyl-like C2 and C1 linkers, rather than C3. C2- and C3-linkers are preferred as single-base length analogs. Larger anucleosidic spacers are contemplated herein having stretches of a few C2 or C3 linkers, or by incorporating a single C6 linker (for a two-base gap), or C9 (for 3 bases), and so forth up C18 linkers, and the like.

As used herein, the phrase "abasic moiety" refers to moieties that approximate the conformation of a

nucleotide but are chemically distinct from nucleotides, which include sugars or sugar analogs without base moieties. For example, an abasic site consists of the sugar-phosphate backbone without the base, such as
5 tetrahydrofuran-phosphate (dSpacer; Glen Research, Sterling, VA), can be used as a nucleotide analog. Other backbones include those in which one or two of the non-bridging oxygen atoms of the phosphate moiety of a nucleotide have been replaced with a sulfur-containing
10 group (especially a phosphorothioate), an alkyl group (especially a methyl or ethyl alkyl group), a nitrogen-containing group (especially an amine), and/or a selenium-containing group. In addition, peptide nucleic acids (PNAs) (Buchardt O, *Trends Biotechnol* 1993
15 Sep;11(9):384-6) or a carbohydrate are alternate backbones that can be used. It is contemplated that abasic modifications of these backbone types can be used as nucleotide analogs.

As used herein, "non-natural base variations"
20 are non-canonical bases often referred to as "degenerate bases" since they exhibit some ability to base pair to any of the 4 standard bases. Exemplary non-natural base variations include, for example, "purine" and "pyrimidine" (which would be the structural scaffolds for
25 A/G and C/T, respectively), as well as fluorine-derivatized bases, and the like. Examples of other non-natural base variations include 5-nitroindole, 3-nitropyrrole, and inosine. Numerous other "natural" base modifications that result from carcinogen exposure are
30 also contemplated herein (see, e.g., Glen Research Catalog). The single nucleotide spacer can also include nucleotide ribose or deoxyribose analogs.

As used herein, the phrase "naturally occurring, non-Watson/Crick, bases" refers to any naturally occurring canonical base other than dA, dC, dG, dT. Common bases would be deoxyinosine (dI) and
5 deoxyuridine (dU). Other naturally occurring base variants can be found in tRNA sequences, and the like.

The single nucleotide spacer can also include non-complementary Watson/Crick nucleotide bases. An example of a mismatched nucleotide base would be the
10 substitution of a thymidine base with an adenine base such that the intra helical base pair would be A:A instead of T:A and hence disrupt base pairing. For example, it is well known that some non-Watson/Crick pairs have moderate stability, such as G/T pairs. For
15 any base pair, there are 6 potential mis-pairs using the standard 4 bases.

As used herein, the term "hybridize", or grammatical variations thereof, refers to the ability of two strands of nucleic acid molecules to hydrogen bond in
20 a sequence dependent manner. For example, under appropriate conditions, complementary nucleotide sequences can hybridize to form double stranded DNA or RNA, or a double stranded hybrid of RNA and DNA. Nucleotide changes in the complementary sequence that
25 disrupt base-pairing result in a decreased stability of the hybrid. These concepts are known in the art and described in Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd ed. (1989), which is incorporated herein by reference.

30 Stringency levels used to hybridize a given probe with target-DNA can be readily varied by those of skill in the art. The phrase "stringent hybridization"

is used herein to refer to conditions under which nucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. In general, the stability of a hybrid is a function of GC content, cation concentration, formamide concentration and temperature. Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions.

As used herein, the phrase "moderately stringent hybridization" refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60% identity, preferably about 75% identity, more preferably about 85% identity to the target DNA; with greater than about 90% identity to target-DNA being especially preferred. Preferably, moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C.

The phrase "high stringency hybridization" refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65°C (i.e., if a hybrid is not stable in 0.018M NaCl at 65°C, it will not be stable under high stringency conditions, as contemplated herein). High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C.

The phrase "low stringency hybridization" refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 42°C, followed by washing in 1X SSPE, 0.2% SDS, at 50°C. Denhart's solution and SSPE (see, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989) are well known to those of skill in the art as are other suitable hybridization buffers.

As used herein, the phrase "immediately adjacent the 3' end" refers to a specific first nucleotide position, typically on the target nucleic acid or on a nucleic acid having SEQ ID NO:4n+4, or complementary sequences thereof, which is immediately 5' of a second nucleotide position on the same nucleic acid, in which the second nucleotide position is complementary to the 3'-end nucleotide of SEQ ID NO:4n+3 or complementary sequence thereof. For example, Figure 2A shows a nucleotide sequence representative of a target nucleic acid sequence or a nucleic acid sequence having SEQ ID NO:4n+4, labeled "Template strand." Figure 2A also shows a nucleotide sequence representative of a nucleic acid sequence having SEQ ID NO:4n+3, labeled "SNPE primer." The 3'-end nucleotide of the SNPE primer is an "A" which is complementary to a "T" on the Template strand. The nucleic acid immediately 5' of the "T" on the Template strand is a "G," and is labeled in the figure "SNP to be typed. Accordingly, the "G" nucleotide on the Template strand, labeled as the SNP to be typed, is "immediately adjacent the 3' end" of the SNPE primer in Figure 2A.

Preferably, the invention oligonucleotides corresponding to SEQ ID NOs:4n+1 and 4n+2, where n=0

through 934 (i.e., columns 1 and 2 of Table 1) can be used as two amplification primers for increasing the amount of target nucleic acids (set forth in column 4 of Table 1) containing the SNP. In addition, in view of the
5 target nucleic acids disclosed herein in column 4 of Table 1, those of skill in art can readily design additional amplification primers that can amplify larger target nucleic acid fragments comprising the target nucleic acids set forth in column 4. Likewise, it is
10 contemplated herein that those of skill in art can readily design additional amplification primers that can amplify smaller target nucleic acid fragments, so long as the smaller target nucleic acid comprises the SNP-site set forth in column 4. The amplification primer pairs
15 are designed such that the primers generate a target nucleic acid strand complementary to the genotyping primer and resistant to degradation, for example, by 5'-3' exonuclease.

As used herein, a nucleic acid that is
20 "resistant to degradation" or "degradation-resistant" is a nucleic acid that is resistant to chemical degradation, acid hydrolysis, base hydrolysis, or other chemical-induced hydrolysis, or is resistant to enzymatic degradation. A nucleic acid that is resistant to
25 enzymatic degradation can be resistant to exonuclease activity, endonuclease activity, and the like. In a preferred embodiment, a nucleic acid that is resistant to degradation is resistant to 5'-3' exonuclease activity, by using phosphorothioation, and the like.

30 A method of using 5'-3' exonuclease in the preparation of target nucleic acids is the subject of Nikiforov et al., U.S. Patent 5,518,900, which is herein incorporated by reference. Examples of 5'-3' exonuclease

resistant nucleic acids are disclosed in Zon, G. et al., (Anticancer Drug Design 6:539-568 (1991)) and Goodchild, J. et al. (Bioconjugate Chem. 1:613-629 (1990)), both of which are incorporated herein by reference in their
5 entirety. In general, suitable degradation-resistant nucleotide derivatives in which one or two of the non-bridging oxygen atoms of the phosphate moiety of a nucleotide have been replaced with a sulfur-containing group (especially a phosphorothioate), an alkyl group
10 (especially a methyl or ethyl alkyl group), a nitrogen-containing group (especially an amine), and/or a selenium-containing group, etc. Other modifications that confer degradation resistance such as 5'-3' exonuclease resistance can be used, for example, peptide nucleic
15 acids (PNAs) (Buchardt, O. *supra*) or 2'-O-methyl ribose modified nucleic acids Srivastava S., C. et al., U.S. Pat. No. 5,214,135. Degradation resistance is preferably achieved by the primer containing a phosphorothioate modification at the 5' end of the nucleic acid and, in a
20 preferred embodiment, containing four phosphorothioate linkages at the 5' end.

The selected nucleotide derivative is suitable for *in vitro* primer-mediated extension and provides
nuclease resistance to the region of the nucleic acid
25 molecule in which it is incorporated. In a preferred embodiment, a nucleotide derivative confers resistance to exonucleases that attack double-stranded nucleic acids from the 5'-end (5'-3' exonucleases). Examples of such exonucleases include bacteriophage T7 gene 6 exonuclease
30 and bacteriophage lambda exonuclease. Both exonucleases are inhibited by the presence of phosphorothioate bonds so as to allow the selected degradation of the unmodified nucleic acid strand. However, any double-strand specific, 5'-3' exonuclease can be used for this process,

provided its activity is affected by the presence of the modified nucleotides. The preferred enzyme when using phosphorothioate derivatives is the T7 gene 6 exonuclease, which shows maximal enzymatic activity in
5 the same buffer used for many DNA dependent polymerase buffers including Taq polymerase. The 5'-3' exonuclease resistant properties of phosphorothioate derivative-containing DNA molecules are discussed, for example, in Kunkel, T.A. et al. (In: Nucleic Acids and Molecular
10 Biology, Vol. 2, 124-135 (Eckstein F. et al., eds.), Springer-Verlag, Berlin, (1988)). The 3'-5' exonuclease resistant properties of phosphorothioate nucleotide containing nucleic acid molecules are disclosed in Putney et al. (Proc. Natl. Acad. Sci. (U.S.A.) 78:7350-7354
15 (1981)) and Gupta A.P. et al. (Nucl. Acids Res., 12:5897-5911 (1984)).

The amplification primers can be used to generate amplified nucleic acid products by any *in vitro* amplification method known to those skilled in the art
20 that uses a DNA dependent or RNA dependent DNA or RNA polymerase. The preferred method is the polymerase chain reaction (PCR) that involves template-dependent extension using thermally stable DNA polymerase (Mullis K et al, Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986);
25 Erlich H et al., EP 50,424; EP 84,796, EP 258,017, EP 237-362; Mullis K., EP 201,184; Mullis, K. et al, U.S. Pat. No. 4,683,202; Erlich, H., U.S. Pat. No. 4,582,788; and Saiki, R. et al., U.S. Pat. No. 4,683,194), incorporated herein by reference. PCR achieves the amplification of a
30 specific nucleic acid sequence using two oligonucleotide primers complementary to regions of the sequence to be amplified. Extension products incorporating primers then become templates for subsequent amplification steps. Reviews of the polymerase chain reaction are provided by

Mullis, K.B., (*supra*); Saiki, R.K. et al., (Bio/Technology 3:1008-1012 (1985)); and Mullis, K.B. et al. (Meth. Enzymol. 155:335-350 (1987)), which is incorporated herein by reference.

5 Other nucleic acid amplification procedures can be used and include transcription-based amplification systems (Kwoh, D. et al., Proc. Natl. Acad. Sci. (U.S.A.) 86:1173 (1989)); Gingeras T.R. et al., PCT appl. WO 88/10315 (priority: U.S. patent application Ser. Nos. 10 064,141 and 202,978); Miller, H.I. et al., PCT appl. WO 89/06700 (priority: U.S. patent application Ser. No. 146,462); Davey, C. et al., (European Patent Application Publication No. 329,822)), RNA-dependent RNA amplification (Q β replicase (Kramer, F.R. et al., U.S. 15 Pat. No. 4,786,600), and ligation-based amplification systems (Wu, D.Y. et al., Genomics 4:560 (1989)).

 Amplification primers can be any length but are preferably as long as 90 nucleotides, preferably between 14 to 50 nucleotides, more preferably between 17 and 30 20 nucleotides.

 In accordance with another embodiment of the invention, methods are provided for genotyping a nucleic acid sample comprising hybridizing an invention oligonucleotide to the nucleic acid sample, and 25 preferably, performing a primer extension reaction .

 As used herein, the term "genotyping" refers to determining the presence, absence or identity of a nucleotide base (e.g., a SNP) at a specific position in a target nucleic acid. First, a sample containing the 30 target nucleic acid is treated, if such nucleic acid is double-stranded, so as to obtain unpaired nucleotide

bases spanning the specific position. If the target nucleic acid is single-stranded, this step is not necessary. Second, the sample containing the target nucleic acid is contacted with a genotyping oligonucleotide (also referred to as a genotyping primer) under hybridizing conditions. The genotyping oligonucleotide is capable of hybridizing with a stretch of nucleotide bases present in the target nucleic acid, adjacent the nucleotide base to be identified (e.g., a SNP), so as to form a duplex between the genotyping oligo and the target nucleic acid. When the genotyping oligonucleotide is "immediately adjacent" the nucleotide base to be identified (e.g., a SNP), the genotyping oligonucleotide hybridizes with the target nucleic acid in such a way that either the 3' or 5' end of the genotyping oligonucleotide is complementary to a nucleotide on the target nucleic acid that is located immediately 5' or 3', respectively, of the nucleotide base to be identified (see, e.g., Figure 2A and 2B). It is also contemplated herein that the invention genotyping oligos can be fragments of the oligos in column 3 of the Table 1 (i.e., SEQ ID NO:4n+3, n=0-934) hybridizable to the target nucleic acid and adjacent to the nucleotide base to be identified such that the 3' end of the genotyping oligo is 1 up to 10, preferably 3 up to 6, nucleotides upstream from the nucleotide base to be identified in the target nucleic acid.

As used herein, the phrase "primer extension" refers to enzymatic extension of the genotyping primer in the resultant duplex by one or more nucleotides, catalyzed, for example, by a DNA polymerase, and the like. Such primer extension thus depends on correct base pairing of the added nucleotide to the nucleotide base to be identified.

In one embodiment, the duplex of genotyping primer and target nucleic acid can then be contacted with a reagent containing at least two, but preferably four terminators, at least one of which terminators being
5 labeled. The duplex of genotyping primer and the target nucleic acid is contacted with the reagent under conditions permitting base pairing of a complementary terminator present in the reagent with the nucleotide base to be identified and the occurrence of a
10 template-dependent, primer extension reaction so as to incorporate a terminator at the 3' end of the primer. The net result is that the genotyping primer has been extended by one terminator. Next, the presence or absence of a labeled terminator at the 3' end of the
15 extended genotyping primer is detected. The identity of the labeled terminator indicates which terminator has base paired to the next base in the target nucleic acid. Since the terminator is complementary to the next base in the target nucleic acid, the identity of the next base in
20 the target nucleic acid is thereby determined.

It is also contemplated that the genotyping primer can be extended by a nucleic acid template-dependent ligation reaction. In such a reaction, a ligating oligonucleotide is used that hybridizes to the
25 target nucleic acid only if the nucleotide residue corresponding to the SNP on the target molecule is complementary to the corresponding nucleotide on the ligating oligonucleotide. If such a ligating oligonucleotide hybridizes with the target nucleic acid,
30 the ligating oligonucleotide and the genotyping oligonucleotide will be immediately adjacent each other. The genotyping primer and ligating primer can then be ligated by contacting the target nucleic acid, genotyping

primer and ligating oligonucleotide with a nucleic acid ligase. The ligation reaction can ligate the 5' end of the genotyping primer to the 3' end of the ligating primer, or alternatively, the ligation reaction can
5 ligate the 3' end of the genotyping primer to the 5' end of the ligating primer. Either the genotyping primer or the ligating oligonucleotide can be labeled with a detectable marker. The detection of a ligation product indicates the presence of the SNP nucleotide targeted by
10 the ligation primer.

As used herein, a detectable marker is any molecule or structure that can be detected by spectroscopic, scattering, emission, absorption, binding, or other known detection methods known in the art.

15 Exemplary detectable markers include a radionuclide, a fluorochrome, a colorimetric agent, a magnetic substance, an electron-rich material such as a metal, a luminescent tag, or a detectable binding agent such as biotin. Preferably, the detectable marker is a fluorochrome or a
20 detectable binding agent. More preferably, the detectable marker is a fluorescent dye or a detectable binding agent that can be bound by an antibody. In one embodiment, the detectable marker is biotin or fluorescein. A detectable binding agent refers to any
25 substance that can bind a solid support, a probe molecule, or other molecule that binds the detectable binding agent and, in turn, is then detected or permits identification of the nucleic acid which is labeled with the detectable binding agent. For example, a detectable
30 marker can be biotin which is bound by an antibody specific to biotin, whereupon the antibody can then be detected in an ELISA-based assay. Other detectable markers known in the art may be used in the invention assay, for example, markers listed in the catalog of

Molecular Probes (Eugene, OR), markers listed in the catalog of Synthesgen (Houston, TX), and markers listed in WO 98/59066, which are all herein incorporated by reference.

5 It is further contemplated herein that one or more primer extension reactions, used in genotyping a nucleic acid sample comprising one or more different target nucleic acids, can be carried out in the same reaction vessel. In one embodiment, at least one
10 terminator is labeled with a detectable marker. In another embodiment, one or more genotyping primers are each labeled with a detectable marker. Detection of identity of the SNPs on the one or more target nucleic acids can then be carried out using one of a variety of
15 methods including spectroscopic detection of one or more detectable markers, separation of one or more nucleic acid sequences used to identify the identity of the corresponding one or more SNPs. Such methods for the detection of one or more SNPs in a single reaction vessel
20 are described in WO 98/59066, which is herein incorporated by reference.

 In a preferred embodiment, the primer extension reaction is a "single-nucleotide primer extension" as described, for example in United States Patent No.
25 5,679,524 and in United States Patent No. 5,888,819, incorporated herein by reference in their entirety. In this method, a genotyping primer hybridizes with the target nucleic acid immediately adjacent the nucleotide base to be identified, so as to form a duplex between the
30 genotyping primer and the target nucleic acid such that the nucleotide base to be identified is the first unpaired base in the template target nucleic acid immediately downstream of the 3' end of the genotyping

primer in the duplex of genotyping primer and target nucleic acid. In this embodiment, primer extension will add a single terminator residue which is complementary to the base to be determined in the target nucleic acid.

- 5 Identification of the terminator will thus provide identification of the base on the target nucleic acid to be determined.

Typically, two or more terminators are labeled, each labeled with a different detectable marker, thereby
10 permitting simultaneous detection (and hence, identification) of the two or more labeled terminators. Preferably four terminators are labeled, each with a different detectable marker, thereby permitting simultaneous detection and identification of up to four
15 labeled terminators.

In the embodiment in which only one terminator is labeled (a first labeled terminator), the above-described primer extension procedure can be repeated at least once, using a second labeled terminator that is
20 different from the first labeled terminator. This process can be repeated once, twice or three times, using a different, labeled terminator for each repetition.

As used herein, the phrase "nucleic acid sample" refers to one or more of target nucleic acids,
25 which can be from any source. The sample of target nucleic acids can be natural or synthetic (i.e., synthesized enzymatically in vitro). The sample of target nucleic acids can comprise deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs), or copolymers of
30 deoxyribonucleic acid and ribonucleic acid. The target nucleic acid can be a deoxyribonucleic acid (DNA), a ribonucleic acid (RNA), or a copolymer of

deoxyribonucleic acid and ribonucleic acid. The target nucleic acid can be synthesized enzymatically in vivo, synthesized enzymatically in vitro, or synthesized non-enzymatically. The sample containing the target
5 nucleic acid or acids can comprise genomic DNA from an organism, RNA transcripts thereof, or cDNA prepared from RNA transcripts thereof. The sample containing the target nucleic acid or acids can also comprise
extragenomic DNA from an organism, RNA transcripts
10 thereof, or cDNA prepared from RNA transcripts thereof. Preferably, the target nucleic acid or acids are synthesized by amplification methods described herein. More preferably, the target nucleic acid or acids are synthesized by the polymerase chain reaction.

15 The sample can be taken from any organism, but is preferably human. Some examples of organisms to which the method of the subject invention is applicable include plants, microorganisms, viruses, birds, vertebrates, invertebrates, mammals, humans, horses, dogs, cows, cats,
20 pigs, or sheep. For assay of genomic DNA, virtually any biological tissue samples can be used, including whole blood, semen, saliva, tears, urine, fecal material, sweat, buccal, skin and hair. Genomic DNA is typically amplified by PCR, and the like, before analysis.

25 The target nucleic acid can comprise one or more moieties that permit affinity separation of the target nucleic acid from the unincorporated reagent and/or the primer. The target nucleic acid can comprise biotin which permits affinity separation of the target
30 nucleic acid from the unincorporated reagent and/or the primer via binding of the biotin to streptavidin which is attached to a solid support. The sequence of the target nucleic acid can comprise a DNA sequence that permits

affinity separation of the target nucleic acid from the unincorporated reagent and/or the primer via base pairing to a complementary sequence present in a nucleic acid attached to a solid support. The target nucleic acid can
5 be labeled with a detectable marker; this detectable marker can be different from any detectable marker present in the reagent or attached to the primer.

In a preferred embodiment, the target nucleic acid sequence is synthesized by an amplification method
10 and is modified at the 5' end to prevent hydrolysis by, for example, exonuclease activity, as described herein. See, for example, United States Patent 5,518,900, which describes a method for generating single-stranded nucleic acid molecules containing nuclease-resistant nucleotides.

15 The genotyping primer can comprise one or more moieties that permit affinity separation of the primer from the unincorporated reagent and/or the target nucleic acid. The genotyping primer can comprise biotin which permits affinity separation of the primer from the
20 unincorporated reagent and/or target nucleic acid via binding of the biotin to streptavidin which is attached to a solid support. The sequence of the genotyping primer can comprise a DNA sequence that permits affinity separation of the primer from the unincorporated reagent
25 and/or the target nucleic acid via base pairing to a complementary sequence present in a nucleic acid attached to a solid support.

In another embodiment of the invention, a method is provided for genotyping a nucleic acid sample
30 comprising:

- a) amplifying a target nucleic acid sequence that hybridizes to an oligonucleotide selected from the group of SEQ ID NOs consisting of $4n+3$, or complementary sequences thereof; and
- 5 b) performing a single-nucleotide primer extension reaction employing an oligonucleotide comprising a nucleic acid selected from the group of SEQ ID NOs consisting of $4n+3$, or complementary sequences thereof,
- 10 wherein $n=0$ through 934 and is the same value in both steps a and b.

As used herein, a target nucleic acid sequence that hybridizes to an oligonucleotide selected from the group of SEQ ID NOs consisting of $4n+3$ or complementary

15 sequences thereof refers to a target nucleic acid comprising a sequence substantially similar to at least a portion of nucleic acid of SEQ ID NO: $4n+4$, or complementary sequences thereof that hybridizes to a nucleic acid of SEQ ID NO: $4n+3$. Typically, such a target

20 nucleic acid sequence will comprise the nucleic acid of SEQ ID NO: $4n+4$ with the exception of the nucleotide immediately adjacent the 3' end of the nucleic acid of SEQ ID NO: $4n+3$, which may or may not be present, where n is the same value for both SEQ ID NOs: $4n+3$ and $4n+4$.

25 In a further embodiment of the invention, a kit is provided which contains at least one genotyping primer. As used herein, a kit refers to a genotyping system in kit form, comprising at least one invention genotyping primer, and optionally further comprising

30 chain termination reagent in combination with a DNA polymerase with or without an associated 3' to 5'

exonuclease function, and an appropriate salt and cofactor mixture, in a suitable packaging material. Invention kits are useful for assaying for the presence or absence of a nucleotide at a specified location on one or more target nucleic acids. Preferably, the invention kit is useful for determining the identity of a nucleotide at a specified location on one or more target nucleic acids. To facilitate the assaying for the presence or absence, or determining the identity of the SNP nucleotide on the target nucleic acid, the invention kit provides a genotyping primer, one or more terminators, or both a genotyping primer and one or more terminators, that are labeled with a detectable marker.

The conditions for the occurrence of the template-dependent, primer extension reaction can be created, in part, by the presence of a suitable template-dependent enzyme. Some of the suitable template-dependent enzymes are DNA polymerases. The DNA polymerase can be of several types. The DNA polymerase must, however, be primer and template dependent. For example, *E. coli* DNA polymerase I or the "Klenow fragment" thereof, T4 DNA polymerase, T7 DNA polymerase ("Sequenase"), *Thermus aquaticus* DNA polymerase, or a retroviral reverse transcriptase can be used. RNA polymerases such as T3 or T7 RNA polymerase can also be used in some protocols. Depending upon the polymerase, different solution conditions and different temperature ranges are used for the hybridization and extension reactions.

The reagents of the subject invention typically permit the typing of nucleic acids of interest by facilitating the analysis of the 3' terminal addition of terminators to a specific primer or primers under specific hybridization and primer extension conditions.

Using only one terminator in the chain termination reagent as the nucleoside triphosphate substrate ensures addition of only one nucleotide residue to the 3' terminus of the primer in the polymerase reaction. Using
5 all four terminators simultaneously ensures fidelity, i.e., suppression of misreading.

A genotyping primer of an invention kit comprises a genotyping primer as described above, which is capable of hybridizing to a target nucleic acid. An
10 invention kit comprises one or more genotyping primers. In one embodiment of an invention kit, the genotyping primer(s) comprises any of SEQ ID NO:4n+3, or the complement thereof, where n=0 to 934. In another embodiment the genotyping primer(s) comprises a portion
15 of any of SEQ ID NO:4n+4, or the complement thereof, where n=0 to 934. In yet another embodiment the genotyping primer(s) hybridizes with any of SEQ ID NO:4n+4, or the complement thereof, where n=0 to 934.

A suitable kit includes at least one invention
20 genotyping primer, and optionally further comprising chain termination reagent in combination with a DNA polymerase with or without an associated 3' to 5' exonuclease function, and an appropriate salt and cofactor mixture, as a separately packaged chemical
25 reagent(s) in an amount sufficient for at least one assay. Instructions for use of the packaged reagent are also typically included. Those of skill in the art can readily incorporate invention genotyping primers into kit form in combination with appropriate buffers and
30 solutions for the practice of the invention methods as described herein.

In another embodiment, an invention kit comprises a genotyping primer and two amplification primers. This kit can also comprise chain termination reagent in combination with a DNA polymerase with or without an associated 3' to 5' exonuclease function, and an appropriate salt and cofactor mixture to be used in conjunction with the genotyping primer, and can also comprise nucleotide reagent in combination with a DNA polymerase with or without an associated 3' to 5' exonuclease function, and an appropriate salt and cofactor mixture to be used in conjunction with the amplification primers, in a suitable packaging material. Such invention kits are useful for (1) amplifying one or more target nucleic acid sequences in a nucleic acid sample and (2) assaying for the presence or absence of a nucleotide at a specified location on one or more target nucleic acids. Preferably, invention kits are useful for (1) amplifying one or more target nucleic acid sequences in a nucleic acid sample and (2) determining the identity of a nucleotide at a specified location on one or more target nucleic acids.

Preferably, a kit comprising one genotyping primer and two amplification primers comprises a genotyping primer that hybridizes to the sequence SEQ ID NO:4n+4 and amplification primers of the sequence SEQ ID NOs:4n+1 and 4n+2, where n=0 through 934 and the three oligonucleotides correspond to SEQ ID NOs having the same value for n.

More preferably, a kit comprising one genotyping primer and two amplification primers comprises a genotyping primer of the sequence SEQ ID NO:4n+3 and amplification primers of the sequence SEQ ID NOs:4n+1 and 4n+2, where n=0 through 934 and the three

oligonucleotides correspond to three consecutive SEQ ID NOs having the same value for n.

In yet another embodiment of the invention, a kit comprises two amplification primers. This kit can
5 also comprise nucleotide reagent in combination with a DNA polymerase with or without an associated 3' to 5' exonuclease function, and an appropriate salt and cofactor mixture to be used in conjunction with the amplification primers, in a suitable packaging material.
10 Such invention kits are useful for amplifying one or more target nucleic acid sequences in a nucleic acid sample.

A termination reagent of an invention kit refers to a reagent comprising at least one terminator. Preferably, a termination reagent comprises two
15 terminators. More preferably, a termination reagent comprises three terminators. Most preferably, a termination reagent comprises four terminators. A termination reagent will comprise one or more terminators labeled with a detectable marker. Preferably, a
20 termination reagent comprises two or more terminators labeled with a detectable marker. More preferably, a termination reagent comprises three or more terminators labeled with a detectable marker. Most preferably, a termination reagent comprises four or more terminators
25 labeled with a detectable marker.

A nucleotide reagent of an invention kit for use in an amplification reaction refers to a reagent comprising at least one nucleotide triphosphate. Preferably, a nucleotide reagent comprises two nucleotide
30 triphosphates. More preferably, a nucleotide reagent comprises three nucleotide triphosphates. Most

preferably, a nucleotide reagent comprises four nucleotide triphosphates.

In a particularly preferred embodiment, an invention kit comprises a collection reagents termed

5 "Master Mix" as described in Example I. Exemplary Master Mix 2x is as follows: 768 mL of sterile, distilled water 200 mL of 10xPCR salts (100 mM Tris-HCl, pH 8.5, 500 mM KCl, 15 mM MgCl₂ and 0.01% Gelatin) and 8 mL of each dNTP stock (100 mM stocks of deoxyribonucleotide triphosphates

10 dATP, dCTP, dGTP, and dTTP) in sterile, distilled H₂O. The final composition being: 50 mM Tris-HCl, pH 8.5, 100 mM KCl, 3 mM MgCl₂, 0.002% Gelatin, and 800 μM each dNTP. This solution can be stored at -20°C until use. Master mix can be prepared in a variety of different

15 concentrations, described as a function of x, as the above example depicts 2x Master Mix. The concentration of the master mix employed herein may vary from 0.5x, 1x, 2x, or 5x, to as high as 10x, 20x, or 50x. Preferably, the master mix is from 2x to 5x.

20 In addition, in place of Tris-HCl, any other buffer which does not interfere with the enzymatic and/or detection processes of required to detect the presence, absence or identity of a SNP may be used. Such buffers include the so-called "Good buffers", and other organo-

25 amino based buffers, and the like. Similarly, the pH may be adjusted to any range capable of carrying out the desired processes. The pH may range from as low as about 6.0, 7.0, or 8.0, to as high as about 8.5, 8.7, or 9.0. Preferably, the pH ranges from about 7.0 to about 8.7.

30 More preferably, the pH ranges from about 8.0 to about 8.5. Buffer concentration may vary from as low as 10 mM, 25 mM or 50 mM, to as high as 75 mM, 100 mM or 200 mM.

Preferably, the buffer concentration is from 25 mM to 100 mM.

In place of KCl, other monovalent-cation salts can be used. Other exemplary monovalent-cation salts include salts with the cation: NH_4^+ , alkalai metal ions Li^+ , Na^+ , Rb^+ , Cs^+ , and other monovalent cations. The anion can be any anion which does not adversely influence the amplification reaction. Exemplary anions are halide anions, F^- , Cl^- , Br^- , I^- , and other anions such as phosphate, sulfate, nitrate, and the like. The concentration of the monovalent salt can be as low as 15 mM, 40 mM, or 75 mM, to as high as 100 mM, 150 mM or 250 mM. Preferably, the monovalent-cation salt concentration is from about 75 to 150 mM.

In place of MgCl_2 , other divalent-cation salts can be used. Other exemplary divalent-cation salts include salts with the cation: Mg^{2+} , Ca^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , and other divalent cations. The anion can be any anion which does not adversely influence the amplification reaction. Exemplary anions are halide anions, F^- , Cl^- , Br^- , I^- , and other anions such as phosphate, sulfate, nitrate, and the like. The concentration of the divalent salt can be as low as 0.5 mM, 1 mM, or 3 mM, to as high as 5 mM, 10 mM or 25 mM. Preferably, the divalent-cation salt concentration is from about 1 mM to 10 mM.

In place of gelatin, other volume exclusion agents can be used such as polyvinyl pyrrolidone, polyethylene glycol, polyacrylamide, linear polyacrylamide, and the like. The volume exclusion reagent can be absent, or can be as low as 0.0001%, 0.0005% or 0.001%, to as high as 0.002%, 0.005% or 0.02%.

Preferably, the volume exclusion agent is about 0.001% to 0.005%.

The concentration of each of dCTP, dGTP, dATP and dTTP (i.e., the dNTPs) can vary in concentration from
5 250 μ M, 500 μ M, or 800 μ M, to 1 mM, 1.5 mM or 2.5 mM.
Preferably, the concentration of the dNTPs is about 500 μ M to 1 mM.

As employed herein, the phrase "packaging material" refers to one or more physical structures used
10 to house the contents of the kit, such as invention primers, and the like. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. The packaging material has a label which indicates that the invention
15 probes can be used for determining the presence or absence of, or preferably the identity of, a particular nucleotide on a target nucleic acid, thereby genotyping the target nucleic acid. In addition, the packaging material contains instructions indicating how the
20 materials within the kit are employed to determine the presence or absence of, or preferably the identity of, a particular nucleotide on a target nucleic acid.

The packaging materials employed herein in relation to diagnostic systems are those customarily
25 utilized in nucleic acid-based assay systems. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding within fixed limits an isolated nucleic acid, oligonucleotide, or primer of the present
30 invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated nucleic acid, oligonucleotide or primer, or

it can be a microtiter plate well to which microgram quantities of a contemplated nucleic acid probe have been operatively affixed.

"Instructions for use" typically include a
5 tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

10 It is contemplated herein that each of the genotyping and amplification primers described herein can be used in the invention methods of genotyping nucleic acid samples, for example, to assess by association analysis the genotype of an individual, or group of
15 individuals, having a pathological phenotypic trait suspected of being caused by one or more single nucleotide polymorphisms. Phenotypic traits suitable for association analysis include diseases that have known but yet unmapped genetic components, e. g.,
20 agammaglobulinemia, diabetes insipidus, Lesch-Nyhan syndrome, muscular dystrophy, Wiskott-Aldrich syndrome, Fabry's disease, familial hypercholesterolemia, polycystic kidney disease, hereditary spherocytosis, von Willebrand's disease, tuberous sclerosis, hereditary
25 hemorrhagic telangiectasia, familial colonic polyposis, Ehlers-Danlos syndrome, osteogenesis imperfecta, and acute intermittent porphyria, and the like.

Phenotypic traits also include symptoms of, or susceptibility to, multifactorial diseases of which a
30 component is or may be genetic, such as autoimmune diseases, inflammation, cancer, system, diseases of the nervous system and infection by pathogenic

microorganisms. Some examples of autoimmune diseases include rheumatoid arthritis, multiple sclerosis, diabetes (insulindependent and non-independent), systemic lupus erythematosus and Graves disease, and the like.

- 5 Some examples of cancers include cancers of the bladder, brain, breast, colon, esophagus, kidney, oral cavity, ovary, pancreas, prostate, skin, stomach, leukemia, liver, lung, and uterus, and the like.

Phenotypic traits also include characteristics
10 such as longevity, appearance (e. g., baldness, obesity), strength, speed, endurance, fertility, and susceptibility or receptivity to particular drugs or therapeutic treatments.

Such correlations can be exploited in several
15 ways. In the case of a strong correlation between a polymorphic form and a disease for which treatment is available, detection of the polymorphic form set in a human or animal patient may justify immediate
administration of treatment, or at least the institution
20 of regular monitoring of the patient. Detection of a polymorphic form correlated with serious disease in a couple contemplating a family may also be valuable to the couple in their reproductive decisions. For example, the female partner might elect to undergo in vitro
25 fertilization to avoid the possibility of transmitting such a polymorphism from her husband to her offspring. In the case of a weaker, but still statistically significant correlation between a polymorphic set and human disease, immediate therapeutic intervention or
30 monitoring may not be justified. Nevertheless, the patient can be motivated to begin simple life-style changes (e. g., diet, exercise) that can be accomplished at little cost to the patient but confer potential benefits in reducing the risk of conditions to which the

patient may have increased susceptibility by virtue of variant alleles. After determining polymorphic form(s) present in an individual at one or more polymorphic sites, this information can be used in a number of
5 methods.

In addition, determination of which polymorphic forms occupy a set of polymorphic sites in an individual identifies a set of polymorphic forms that distinguishes the individual. See generally, National Research
10 Council, The Evaluation of Forensic DNA Evidence (Eds. Pollard et al., National Academy Press, DC, 1996). Thus, each of the genotyping and amplification primers described herein can be used in the invention methods of genotyping nucleic acid samples, for example, to identify
15 a distinguishing or unique set of forensic markers in an individual useful for forensic analysis. For example, one can determine whether a blood sample from a suspect matches a blood or other tissue sample from a crime scene by determining whether the set of polymorphic forms
20 occupying selected polymorphic sites is the same in the suspect and the sample. If the set of polymorphic markers does not match between a suspect and a sample, it can be concluded (barring experimental error) that the suspect was not the source of the sample. If the set of
25 markers does match, one can conclude that the DNA from the suspect is consistent with that found at the crime scene. If frequencies of the polymorphic forms at the loci tested have been determined (e. g., by analysis of a suitable population of individuals), one can perform a
30 statistical analysis to determine the probability that a match of suspect and crime scene sample would occur by chance (see, e.g, WO 95/12607). If several polymorphic loci are tested, the cumulative probability of non-identity for random individuals becomes very high (e. g.,

one billion to one). Such probabilities can be taken into account together with other evidence in determining the guilt or innocence of the suspect.

In addition, each of the genotyping and
5 amplification primers described herein can be used in the invention methods of genotyping nucleic acid samples, for example, to identify a distinguishing or unique set of markers in an individual useful for paternity analysis. The object of paternity testing is usually to determine
10 whether a male is the father of a child. In most cases, the mother of the child is known and thus, the mother's contribution to the child's genotype can be traced. Paternity testing investigates whether the part of the child's genotype not attributable to the mother is
15 consistent with that of the putative father. Paternity testing can be performed by analyzing sets of polymorphisms in the putative father and the child. If the set of polymorphisms in the child attributable to the father does not match the putative father, it can be
20 concluded, barring experimental error, that the putative father is not the real father. If the set of polymorphisms in the child attributable to the father does match the set of polymorphisms of the putative father, a statistical calculation can be performed to
25 determine the probability of coincidental match (see, e.g., WO 95/12607). If several polymorphic loci are included in the analysis, the cumulative probability of exclusion of a random male is very high. This probability can be taken into account in assessing the
30 liability of a putative father whose polymorphic marker set matches the child's polymorphic marker set attributable to his/her father.

The single nucleotide polymorphisms set forth in column 4 of Table 1 (also referred to herein as SEQ ID NO:4n+4, wherein n=0 through 934) may contribute to the phenotype of an organism in different ways. Some of these polymorphisms may occur within a protein coding sequence and contribute to phenotype by affecting protein structure. The effect may be neutral, beneficial or detrimental, or both beneficial and detrimental, depending on the circumstances. For example, a heterozygous sickle cell mutation confers resistance to malaria, but a homozygous sickle cell mutation is usually lethal. Others of these polymorphisms may occur in noncoding regions but may exert phenotypic effects indirectly via influence on replication, transcription, and translation. A single polymorphism may affect more than one phenotypic trait. Likewise, a single phenotypic trait may be affected by polymorphisms in different genes. Further, some of these polymorphisms may predispose an individual to a distinct mutation that is causally related to a certain phenotype.

Phenotypic traits include diseases that have known but yet unmapped genetic components. Phenotypic traits also include symptoms of, or susceptibility to, multifactorial diseases of which a component is or may be genetic, such as autoimmune diseases, inflammation, cancer, diseases of the nervous system, and infection by pathogenic microorganisms. Some examples of autoimmune diseases include rheumatoid arthritis, multiple sclerosis, diabetes (insulin-dependent and non-independent), systemic lupus erythematosus and Graves disease. Some examples of cancers include cancers of the bladder, brain, breast, colon, esophagus, kidney, leukemia, liver, lung, oral cavity, ovary, pancreas, prostate, skin, stomach and uterus. Phenotypic traits

also include characteristics such as longevity, appearance (e. g., baldness, obesity), strength, speed, endurance, fertility, and susceptibility or receptivity to particular drugs or therapeutic treatments.

5 Correlation is performed for a population of individuals who have been tested for the presence or absence of a phenotypic trait of interest and for polymorphic markers sets. To perform such analysis, the presence or absence of a single or a set of polymorphisms
10 (i. e. a polymorphic set) is determined for a set of the individuals, some of whom exhibit a particular trait, and some of which exhibit lack of the trait. The alleles of each polymorphism of the set are then reviewed to determine whether the presence or absence of a particular
15 allele is associated with the trait of interest. Correlation can be performed by standard statistical methods such as a chi-squared test and statistically significant correlations between polymorphic form(s) and phenotypic characteristics are noted. For example, it
20 might be found that the presence of allele A1 at polymorphism A correlates with heart disease. Such correlations can be exploited in several ways as set forth above.

 For example, in the case of a strong
25 correlation between a set of one or more polymorphic forms and a disease for which treatment is available, detection of the polymorphic form set in a human or animal patient may justify immediate administration of treatment, or at least the institution of regular
30 monitoring of the patient. Thus, the methods of genotyping nucleic acid samples described herein can be used, for example, to diagnose any of more than 3000 genetic diseases currently known or to be identified,

e.g., hemophilias, thalassemias, Duchene Muscular Dystrophy (DMD), Huntington's Disease (HD), Alzheimer's Disease and Cystic Fibrosis (CF), and the like.

It is also contemplated herein that each of the
5 genotyping and amplification primers described herein can be used in the invention methods of genotyping nucleic acid samples, for example, for assessing the pharmacogenomic susceptibility of a subject harboring a single nucleotide polymorphism to a particular
10 pharmaceutical compound, or to a class of such compounds. Genetic polymorphism in drug metabolizing enzymes, drug transporters, receptors for pharmaceutical agents, and other drug targets have been correlated with individual differences based on distinction in the efficacy and
15 toxicity of the pharmaceutical agent administered to a subject. Pharmacogenomic characterization of a subjects susceptibility to a drug enhances the ability to tailor a dosing regimen to the particular genetic constitution of the subject, thereby enhancing and optimizing the
20 therapeutic effectiveness of the therapy.

All U.S. patents and all publications mentioned herein are incorporated in their entirety by reference thereto. The invention will now be described in greater detail by reference to the following non-limiting
25 examples.

Examples

Example I

Preparation of 2x Master Mix for PCR

A component of genotyping using SNPE is accurate and efficient amplification of the nucleic acid region being tested for the SNP. To facilitate control of the PCR used, a reaction mixture, referred to herein as "2x Master Mix," has been produced to achieve more successful PCRs.

2x Master Mix was prepared as follows: 10xPCR salts was 100 mM Tris-HCl, pH 8.5, 500 mM KCl, 15 mM MgCl₂ and 0.01% Gelatin, stored in a -20°C freezer. 100 mM stocks of deoxyribonucleotide triphosphates dATP, dCTP, dGTP, and dTTP (dNTPs) (LTI, Bethesda, MD) were made in sterile, distilled H₂O. To 768 mL of sterile, distilled water 200 mL of 10xPCR salts and 8 mL of each dNTP stock was added. The final composition was: 50 mM Tris-HCl, pH 8.5, 100 mM KCl, 3 mM MgCl₂, 0.002% Gelatin, and 800 µM each dNTP. The solution was stored at -20°C until use.

20

Example II

Single Nucleotide Polymorphism Detection by Single Nucleotide Primer Extension (SNPE)

Single nucleotide polymorphisms are the most common form of genetic diversity and are thought to be direct and indirect markers of many human diseases. The following example shows the use of Single Nucleotide

Primer Extension (SNPE) to identify the presence of SNPs. The method of SNPE is the subject of US Patent 5,888,819 and more recently described in detail by Reynolds et al. in *DNA Markers: Protocols, Applications, and Overviews* (ed. G. Caetano-Anolles), pp. 199-211, Wiley-Liss, New York, NY (1997), both of which are herein incorporated by reference.

The amplification primers listed herein (Table 1, columns 1 and 2) were selected to amplify the corresponding SNP-containing oligo in column 4, forming a PCR amplified target nucleic acid such that the primers do not overlap the corresponding genotyping (SNPE) primer in column 3.

SNPE primers were designed to be complementary to the phosphorothioate-modified strand of the PCR product target nucleic acid and ending one nucleotide base short 3' to the polymorphic site to be interrogated as described (Reynolds et al., *supra*). Either DNA strand can be a target for the SNPE primer as long as the phosphorothioate-modified strand of the PCR product is the complementary strand.

All oligonucleotides were synthesized by standard phosphoramidite chemistry on a PE Biosystems 392/394 DNA Synthesizer using Glen Research (Sterling, VA) reagents. Tetraethylthiuram sulfide (TETD; PE Biosystems, Foster City, CA) was used for phosphorothioate modification as per manufacturer's instructions. All oligonucleotides were deprotected in concentrated ammonia and desalted using NAP5 or NAP25 gel filtration columns (Amersham Pharmacia Biotech, Piscataway, NJ) as per manufacturer's instructions.

Genomic DNA from genetically diverse human individuals purchased from the Coriell Institute for Medical Research (Camden, NJ) was the source of DNA for PCR amplifications. The DNA sample was diluted with
5 sterile, distilled H₂O to a final concentration of 5 ng/μL before use.

PCR amplification of 10 ng of template genomic DNA was performed in 384-well plates under the following conditions: 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 1.5 mM
10 MgCl₂, 0.001% gelatin, 400 μM each of dATP, dCTP, dGTP, and dTTP, (from the 2x Master Mix) and 0.050 U/μL Platinum Taq (*Thermus aquaticus*) DNA polymerase, (LTI, Bethesda, MD) using 0.5 μM each primer concentration in a total volume of 5 μL. The PCR amplification was
15 performed in a PTC-225 DNA Engine Tetrad thermocycler (MJ Research, Watertown, MA) using the following protocol: following a 2 minute denaturation step at 95°C thirty-five cycles were carried out, each consisting of denaturation (30 seconds at 94°C), annealing (2 minutes
20 at 55°C), and extension (30 seconds at 72°C) steps. This was followed by a final extension step (1 min at 72°C) and hold (4°C).

Single stranded target nucleic acid DNA was prepared from the double-stranded PCR product by
25 treatment with a 5'-3' DNA exonuclease. Briefly, 5 μL T7 gene 6 exonuclease (United States Biologicals, Swampscott, MA) in 0.5 M Tris-HCl, pH 7.5, 1 mM Dithiothreitol (DTT), and 0.01% acetylated Bovine Serum Albumin (BSA) was added to a final concentration of 0.45
30 U/μL and incubated for one hour at room temperature. The reaction was terminated by the addition of 5 μL of 3 x SNPE Salt (4.5M NaCl, 30 mM EDTA, and 3 mM cetyltrimethylammonium bromide (CTAB)). The DNA created

by PCR originating from the phosphorothioated primer is protected from nuclease digestion whereas the opposite strand with the unmodified primer is digested and hence will not interfere with the hybridizing strand to the
5 immobilized genotyping (SNPE) primer.

The SNPE primer of interest was attached to a polystyrene 384-well plate (NUNC). Briefly, 20 μ L aliquots of 0.25 μ M SNPE oligonucleotide in 50 mM N,N-dimethyloctylamine hydrochloride, pH 7.0 (ODA; Sigma-
10 Aldrich, Milwaukee, WI) was added to each well and incubated overnight at 37°C. The plates were washed with TNTw (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween-20) three times and once with 1xTE/ISOH (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% isopropanol). The plates were
15 incubated upside down at 50°C for 30 minutes prior to storage in sealed pouch with dessicant. 15 μ L of the exonuclease digestion product was added to the appropriate well and the single-stranded PCR product was allowed to hybridize for 30 minutes at room temperature.
20 All wells were subsequently washed with TNTw three times.

An exonuclease (-) version of the Klenow Fragment of *E. coli* DNA Polymerase I (United States Biologicals, Swampscott, MA) was used for template-directed single nucleotide extension of the SNPE primer.
25 Briefly, the enzyme was diluted in a buffer containing 10 mM Tris-HCl, pH 7.5, 5 mM DTT and 0.5 mg/mL BSA. The extension reaction was performed in 20mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 25mM NaCl, 10 mM MnCl₂, 15 mM Sodium Isocitrate, 1.5 μ M each of the four 2', 3'-
30 dideoxynucleoside 5'-triphosphates (ddNTPs), ddATP, ddCTP, ddTTP, ddGTP. Two of the ddNTPs were unlabeled, one was labeled with biotin, e.g. 2', 3'-dideoxyuridine 5'-triphosphate (biotin-ddUTP), and one labeled with

fluorescein, e.g. fluorescein-labeled 2', 3'-
dideoxycytosine 5'-triphosphate (fluoresceinated-ddCTP).
The determination of which labeled ddNTPs to use depends
on the nucleotide identity of the presumed SNP. For
5 example, a suspected C to T mutation can be assayed using
a mix of ddATP, fluorescein-ddCTP, ddGTP, and biotin-
ddUTP. The enzyme concentration was 0.02 U/ μ L and the
reaction was performed at room temperature for 30
minutes. The plates were washed with TNTw three times,
10 then 0.2N NaOH, and then three times with TNTw.

Detection of the extended primers was performed
by standard ELISA techniques using two nucleotide-
specific labeled antibody conjugates. The dilutions of
each are determined empirically for each antibody lot to
15 achieve approximately equal intensity for the two
colorimetric signals in the presence of both alleles.
Briefly, the wells were incubated for 30 minutes at room
temperature with 10 μ L of 1% fraction V BSA (Sigma, St.
Louis, MO) in TNTw containing an alkaline phosphatase
20 conjugate of antifuorescein (1:2000 dilution)
(Boehringer Mannheim, Indianapolis, IN). After washing
with TNTw six times, the presence of alkaline phosphatase
was determined first by the addition of 25 μ L per well of
a 1.5 mg/mL solution of p-nitrophenyl phosphate (Moss,
25 Pasadena, MD) in 100 mM diethanolamine, pH 9.5, 20 mM
MgCl₂. The plate was immediately placed in a microplate
reader (ICN, Costa Mesa, CA) and the development of color
monitored spectrophotometrically at a wavelength of 405
nm after 24 minutes in an endpoint assay. Next, the
30 detection of biotinylated-ddNTP was performed. The wells
were incubated for 30 minutes at room temperature with 10
 μ L of 1% fraction V BSA in TNTw containing a horseradish
peroxidase-conjugated antibiotin (1:500 dilution) (Zymed,
San Francisco, CA). The plates were then washed three

times with TNTw and the presence of fluorescein was determined by the addition of 25 μ L of a 1 mg/ml solution of tetramethylbenzidine (TMB; Moss, Pasadena, MD). The plate was immediately placed in a microplate reader and
5 the development of color monitored spectrophotometrically at a wavelength of 620 nm after 24 minutes in an endpoint assay.

A scatterplot of the raw data of OD₄₀₅ v. OD₆₂₀ was used in the visual determination of sample genotypes.
10 A polymorphism is identified when three distinct clusters are seen in the positive regions of the scatterplot. These correspond to individuals that are homozygous, forming one cluster that is positive for OD₄₀₅ and another that is positive for OD₆₂₀, or heterozygous, forming a
15 third cluster that is positive for OD₄₀₅ and OD₆₂₀.

Figure 3 shows a scatterplot that was classified polymorphic. The PCR primers used were oligonucleotides of SEQ ID NOS:25 and 26 (Ref No. 261, Table 1). The SNPE primer used was an oligonucleotide
20 with SEQ ID NO:27. The targeted nucleic acid with the suspected polymorphism contains the sequence of SEQ ID NO:28. SNPE was performed using fluorescein-ddCTP and biotin-ddUTP. The Y-axis corresponds to signal obtained from ddUTP incorporation and the X-axis corresponds to
25 signal obtained from ddCTP incorporation. Note that there are clusters where each allele tested is positive (CC or TT) and where both alleles are positive (CT). This is an example where a SNP is identified. Note that there is another cluster about the origin which
30 corresponds to no signal (NS) due to PCR failure.

Table 1 lists the invention oligonucleotides disclosed herein. Each row provides two PCR primers used

to amplify the target DNA comprising a certain SNP
(Columns 1 and 2), one genotyping oligonucleotide (Column
3; referred to as a SNPE primer) used to probe the
identity of the base at the location in the sequence
5 corresponding to that SNP), and sequence of the region
flanking the site of that SNP (Column 4). A "P" listed
to the right of a PCR primer denotes a phosphorothioation
of that primer at its 5' end. In this embodiment, such a
phosphorothioation refers to the five most 5' nucleotides
10 of one of the two PCR primers being linked by four
exonuclease-resistant phosphorothioated linkages (e.g.,
5' C-(p)-G-(p)-C-(p)-A-(p)-GTCTCAGGCCAGCT 3', for SEQ ID
NO:2, where "-(p)-" represents a phosphorothioate
linkage). While phosphorothioation sites are listed for
15 the specific residues herein, it is understood that other
modifications which confer exonuclease resistance can
serve as equally desirable substitutes.

In this particular embodiment, nucleotides
labeled as "X" refer to C3 linkers placed in the
20 corresponding sequences at the location of the X. While
C3 linkers are listed herein, it is understood that other
single-nucleotide-spacers can serve as equally desirable
substitutes.

The sequences of Table 1 are arranged in the
25 same manner as the SEQ ID NOs are listed. Accordingly,
the nucleic acids of SEQ ID NOs:1, 2, 3 and 4 correspond
to the nucleic acids in Row 1, Columns 1, 2, 3 and 4,
respectively. Similarly, the nucleic acids of SEQ ID
NOs:5, 6, 7 and 8 correspond to the nucleic acids in Row

2, Columns 1, 2, 3 and 4, respectively. Therefore, a general formula can be established to describe the SEQ ID NO of each nucleic acid:

$$\text{SEQ ID NO} = (4 \times (\text{Row Number} - 1)) + \text{Column Number}$$

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	2	Modified	3	Modified csa primer	3	Flanking sequence	4
1	255	TTGAAATGTGAATAGTAACTATGTACAGATGA			GGCAGTCTCAGGCGACGT		P	CAGTATGCGAAAAAGACCAACTCGGGAG	P	GTATGGGAAAAAGCAGCTCGGGAGCTCGGGAGCTGCTGTGAGCAGAGAGAGAGTGA		
2	256	CTCTTGGCTAGTTTATGTGAGATGAGA			AGCTTTTCATCTCTCCAAACTTG		P	TGCTACTTTCATCTCTCTCTCTATTA	P	TGCTACTTTCATCTCTCTCTCTATTA		
3	257	CTACTGACTGCTTGACTGAGCTAAATTAAT			AATACATCGAAGACCCCAATCAACA		P	GCTTGACCTCTTGCTATCAATCAAGCC	P	GCTTGACCTCTTGCTATCAATCAAGCC		
4	258	AGTATTAGCTCTTCTCAGGTGAAG			TCAAGGCAAAAGSTATTGCAATATT		P	AAGTCTACGCGCTTCTCAGAAATGAGC	P	AAGTCTACGCGCTTCTCAGAAATGAGC		
5	259	ATCTCAATTCAGCTTTGCTCT			CATGCGGGAATAATCCTTAACTTT		P	CTTTGGAAACAGAGGTCCTCGAGG	P	CTTTGGAAACAGAGGTCCTCGAGG		
6	260	AAGCAGCTCTGACTTTTGCAAT			CCCTCGCAACATAACTCTCAAT		P	GTGTTTCAATAAAGTCAGCAATTTT	P	GTGTTTCAATAAAGTCAGCAATTTT		
7	261	GTGATATAGTAAATATGAGATGGACATTA			ATTTTATGTTTGTGGCTCTGAGTT		P	ATTATATCGAAATAGCTCAGATGAAGA	P	ATTATATCGAAATAGCTCAGATGAAGA		
8	262	CGAGCATCAGGTTATTGAAGC			CTAGGAGTTTACCGCGCT		P	CAGAGAGGCTGTGAAGAGCTACCC	P	CAGAGAGGCTGTGAAGAGCTACCC		
9	263	CAAGTACTAAGGGGCTTG			CTCACAACGCTCTCCATC		P	TTAGAACAACCTGGGGATTACCTTG	P	TTAGAACAACCTGGGGATTACCTTG		
10	265	TGAGGAAGGGGCTGCA			GAAACTCTCTTTCCCAAGAGGA		P	CAAGAGGGGTGCTCTTTTCTACTCC	P	CAAGAGGGGTGCTCTCTTTTCTACTCC		
11	266	GTGGTGAGTGGAGCATGAGTA			ACACAAAGGTTTTTGATGAATC		P	AATGAGATGAGGCTATXAAAGTTTA	P	AATGAGATGAGGCTATXAAAGTTTA		
12	267	TATTTGCCAACTCTTGGC			GAAAAAACAAGGGGTGC		P	AATACCACTATTTGATTCCTGTA	P	AATACCACTATTTGATTCCTGTA		
13	269	AGTGAAGAGGATCRGTCAAGC			CATATAATCAGGACGACAGACAGCA		P	CAGAGAGGCCCAACAATCAGACA	P	CAGAGAGGCCCAACAATCAGACA		
14	270	AGATGCGTGCAGCAGATCACTTAG			AACTCAGACAYTTTAATTCATCAGTA		P	CTCAGATONGAACAATTTTCCCACT	P	CTCAGATONGAACAATTTTCCCACT		
15	271	ATGGTTTAAAGTGTGCGGACTTC			CATCGCAGAAAGTGAAGG		P	ACTTTTGCTCACTCTCTCTCTCTG	P	ACTTTTGCTCACTCTCTCTCTCTG		
16	272	TGACTGCGGATGACAAAGAA			CTATGGCCCTGCATGGCA		P	AGTCACTTAATGCACTGAGGTGAAGC	P	AGTCACTTAATGCACTGAGGTGAAGC		
17	273	TGTACTGCTTTCTCATATGCTCAT			GTTACTACTGTGAGGGGTGGATATAC		P	ATGATATGAGCAAAATTTTACAGTTGAT	P	ATGATATGAGCAAAATTTTACAGTTGAT		
18	274	GAACCTGATTTCTCATGTCACTTATTT			TCTTACTACTATTATGCTGGAACTTCTATAA		P	TTCAATTTAGCAAAATTTTACAGTTGAT	P	TTCAATTTAGCAAAATTTTACAGTTGAT		
19	275	AAATTTGGGTGGGAGCA			CTAAGATATGGAGTGTACCC		P	ATGCAAACTGCAACAACAAGAA	P	ATGCAAACTGCAACAACAAGAA		
20	276	GTGGAACAGATAGACAGACAGAAA			TGCAAGTGTGTGCTCCAACTGCT		P	TTGTTGCCATGTATTTGCTCTTTTC	P	TTGTTGCCATGTATTTGCTCTTTTC		
21	277	ATATAATCATTCAGAGATATTATGCAATG			GCTGGTCAAGGCTGCTT		P	TGCTGTGACACTAGGCTTTGCCCTT	P	TGCTGTGACACTAGGCTTTGCCCTT		
22	278	TCTATGATCTTAAACTCCGCTGTACC			CAATGAATGGGTATGAAGTCT		P	GTGCTGATTTGACACTTTCTGGACCT	P	GTGCTGATTTGACACTTTCTGGACCT		
23	279	GGGTTTACATTTGTGCTTAATCAATAT			TGGGAATAGATAGCGCCACA		P	ATTGCAAAATTTACTKCTACAGCAAT	P	ATTGCAAAATTTACTKCTACAGCAAT		
24	280	CATCTCTACTTTTGGACAGAGT			CCAGCAGTGCAGACATGCG		P	GAATTTTAXATTGATTTATACACTATA	P	GAATTTTAXATTGATTTATACACTATA		
25	281	CTAGTCTTTTGGAAAGATAGCAAA			TCTTGCCCTCTAGTCTTGCAATTC		P	CATGACTCTTAAGTGGATGCAAGATT	P	CATGACTCTTAAGTGGATGCAAGATT		
26	282	ATTAAAGTCCGCTCACTAACTATGCG			CTTGCGGCTCTCAGCGG		P	TGGGACGCTGGGACACAGAAACCCAGATA	P	TGGGACGCTGGGACACAGAAACCCAGATA		
27	283	TGGGTACAGAAACTTACCATTA			ACGCTGCACTCGAGCTG		P	AAAGTGTACTCGCTCTCAAAAAA	P	AAAGTGTACTCGCTCTCAAAAAA		
28	284	TGTACTGCTGCTTAATCACTTTGAG			CCAGATGGATTTTCTGCAAC		P	CACAGAGACTTGAAGTCTTGGAACTC	P	CACAGAGACTTGAAGTCTTGGAACTC		
29	285	AGCAACGAAAAAAAATGTTGTAGA			TGCAATGGATTTTCTAGTGTCC		P	AGGAAAAACAAAGGATATTGCTGTG	P	AGGAAAAACAAAGGATATTGCTGTG		
30	286	CTGGGCAAGGCTTAAACA			CAAAATGCAATACCTTTCCAGTAAC		P	TTGTTGCTTCAAGCCAGACACTTGA	P	TTGTTGCTTCAAGCCAGACACTTGA		
31	287	CAATTTGATGCTTAAACAG			TTTTGGAAATTTTCTAGTGTCC		P	TGTTCCBAACCTTGCAVACTAGG	P	TGTTCCBAACCTTGCAVACTAGG		
32	288	TCAGCATCATTTGTGAGC			TGCTATTCGACTTAAATCGAAGCACAATTT		P	ATGCGAACTGTGAGTCAATGAACCT	P	ATGCGAACTGTGAGTCAATGAACCT		
33	289	CTCAGCCTACAACTTGTATTTAGC			ATA TGGTGGGTGGGACTTAGC		P	AAAGCACAATTTTTCAGAGAGAAGA	P	AAAGCACAATTTTTCAGAGAGAAGA		
34	290	CAGAAAAATAGACATATAGACAGAC			GAAGTTTGGCAGTACAAGTCT		P	CTATAGATACAGATGATATTTATTA	P	CTATAGATACAGATGATATTTATTA		
35	291	ACCTACGATTAACAGGCTCT			CAAGTTTGGCAGTACAAGTCT		P	TGTTGTCACCCCACTCCCTGGGCACTCAGAAATTCATGACGCCCATCTCT	P	TGTTGTCACCCCACTCCCTGGGCACTCAGAAATTCATGACGCCCATCTCT		
36	292	GCTACACTCTTTTGGCTATCAAT			TCAATGTTATTCGATCAATGCTGTGATAG		P	AATGCAAAAATGATATGCTGTGTTG	P	AATGCAAAAATGATATGCTGTGTTG		
37	293	GGGCTTCAGCAAAATACAGCT			CCGGGCACTCTGACTGACA		P	TGCAAAAATGTTTCAATGTTGATTA	P	TGCAAAAATGTTTCAATGTTGATTA		
38	294	ATTAGTCGAGGGCTGG			GGCATCTAGCTGACACATAGC		P	ATAGCCATCAGCCCATCATGACTCC	P	ATAGCCATCAGCCCATCATGACTCC		
39	295	CTTCAAGCTCCACATCACTCTC			AGGTAGGAGAACATGCAAGTTTTTTAA		P	TCCAGAGCGAGTTCTCAGATAAAA	P	TCCAGAGCGAGTTCTCAGATAAAA		
40	296	CATGGGGAGAGAAATCTGATAGTG			TCCAGAGGAGTTCTGATAGAGG		P	GCAGAAATCATCTXTATAGACATTCATTA	P	GCAGAAATCATCTXTATAGACATTCATTA		
41	297	GCATCTGTTTGGAGATTTCATG			ACAGGGAATGATCACTCAGG		P	AAACAAATGTGCTGTAGCTGCGAGGACTCTCTTATCAACACATATTTT	P	AAACAAATGTGCTGTAGCTGCGAGGACTCTCTTATCAACACATATTTT		
42	298	CTCAGCAGCCTCAGCAC			GTATACCAGCGAGGTAGAGAGG		P	AAACCCCTCTACTCCTCAGCTGTGTC	P	AAACCCCTCTACTCCTCAGCTGTGTC		
43	299	AGCACTTAAACCCCTCTATCC			CTTTAAATTCGCAAGAGCA		P	CACACTTTTATAGXATTTTGCCCTCAT	P	CACACTTTTATAGXATTTTGCCCTCAT		
44	300	TACAGGGATTTGGATCTTTTG			CTTTAAATTCAGAGGTTTATAAAGAG		P	CGCAGAGGTGGCCCTCATXAAAGACAT	P	CGCAGAGGTGGCCCTCATXAAAGACAT		
45	301	TTGATCTTACTGAGCTTTCTTCAG			ACCCTGATTAACAGAGGTTTATGAGAG		P	AGATCCAGCTTAGAATTCOCATTTCCA	P	AGATCCAGCTTAGAATTCOCATTTCCA		
46	302	TTAAATGCAAGCACTTTTAAATATAG			TTTTAAGATTTTTCAGAGCGAGTTTC		P	CAGGTTCTTAAAGATAGCCCTTTTGCTCTA	P	CAGGTTCTTAAAGATAGCCCTTTTGCTCTA		
47	303	ATAGAAACAAAGAGATTTGATGTGG			GAATGACTTTTGGTAGGCCA		P	ACCCCTTATAGCAAGCAAGAAATTTCTG	P	ACCCCTTATAGCAAGCAAGAAATTTCTG		
48	304	AATGAGCATGTAGAAGACCC			GTTTGATTCCACATGTAGAAAGGATG		P	CTACTCCXACCCGACAGTGCAGCTTCTG	P	CTACTCCXACCCGACAGTGCAGCTTCTG		
49	305	CTCCTATGCTCGGAAATTAATAGTAAGAA			TCATAACAACATTTTGGCTAAGTCTCT		P	ATTTTAAATGAGTATTGATGCAATA	P	ATTTTAAATGAGTATTGATGCAATA		

Row#	REF#	Upper PCR primer	1	Modified	2	Lower PCR primer	2	Modified GSA primer	3	Flanking sequence
50	305	TAAGTGGGCACTGAGCATG		P	GAATGTGGCCRTTTTATTACATAGTATACA		CCACXKTTGGTTTGGGCKTGACCTCTTT		AGCCCACTTAAGAGARGAATATGCGAAAGAGGTGATGCCCAACCAACCGT	
51	307	TACCAGAAAGTGGCAGATTCT		P	CTTCGAGCCCTCCAAATCTGT		TGCAGATACCTGAAAAATGTGCAAGC	P	TGAGATACCTGAAAAATGTGGAGCAGCTTTAAAAATTTGGGTAAAGGAGCGA	
52	308	TTGATGTTTGTGACAGCTGCT		P	CTATGTAGTTGAACATATGATTTGCGCTTC		TGGCTCTTGGGCTTTGTGACCTTGT	P	TTCCACAGCTGTGTTTGTACACGACNAGGCTCMAAACGACCAAGAGCGA	
53	310	AAATATACAGACAGCTGTTACTGGTG		P	GTGATGGGATATAGGCATCGAGC		GGGAGGTGAGAACAGAGCATAAAGAGTCAGACATATAAGCGCATCTGTGGCTC	P	GGGAGGTGAGAACAGAGCATAAAGAGTCAGACATATAAGCGCATCTGTGGCTC	
54	311	GTGGCACTAAATTAACCTAAMAGCTTC		P	TGTCGTATGCGACAGTTTGAANAATATT		CAAAATAAATCTACCAACAGAGTAA	P	CAAAATAAATCTACCAACAGAGTAAACAGCAACCAACAGAAATGGGACGAAA	
55	312	TTTTCACTATATGGAAGCTAAAC		P	CAATTAACCTCTCTCTCTCTCTCC		CTCTCCCTCTCTCCCAACCATCTCTC	P	CTAGATTGCGTTACCAAGCTTAAAGAGCATGCTGGGAGGCGGACGAG	
56	313	GGCCACACATGATTTCTTAGATC		P	TGATCTGTACACATCGCTGGGCTC		TGCCCGAATTCGATTTTGTGATATA	P	ATATACGAAAAATTTAAATGATTTGTATATACAAAAATCAATTCGACGCA	
57	314	GGCAGATGCCCTCTTACTAGTACTT		P	GTGAATACTAGTAATAGTATAGCATTAAGATGAATGCA		GAATTTTGGCGTCGATTTATAGAGTGTGGAATTCGATTCGATCTTAGTCT	P	GAATTTTGGCGTCGATTTATAGAGTGTGGAATTCGATTCGATCTTAGTCT	
58	315	CTTGCACTAAGAGAGCTTTGTGAAG		P	TCTACTGGAGAGCGTTGTTCTGT		GCTTGATGTCCTTAGTTAGTTACTTCATNT	P	GCTTGATGTCCTTAGTTAGTTACTTCGATNTGATATATGTTCTGACGACAG	
59	316	TTGCCACTGGACACTTTTG		P	AGTCTGGGTCATACGCC		TTAACTGGGTTGTTTGTAGATTTTA	P	TTAACTGGGTTGTTTGTAGATTTTACTGTATATATGTTCTGTTGTT	
60	317	AGAAATTCAGGCTCTGCA		P	CTCTACACAAACACACACTTAAGCA		ATCATATGCGGCTTTTATTTTGGCG	P	ATCATATGCGGCTTTTATTTGGCGCGGCGGCGGCGGCGGCTTATAGT	
61	318	TCTTAAAGAAAGCTCCAG		P	CTCTGGGTACAGAGGCTG		TTAACTGGGTTGTTTGTAGATTTTA	P	TTAACTGGGTTGTTTGTAGATTTTACTGTATATATGTTCTGTTGTT	
62	319	TTCTTACGAAGAGTCTGGTTAC		P	CAGTACAGCATANAACGTTCTCG		AGGTCCACACAGAGCTGCTATTTCT	P	CTGAGTTCCAGTCCACATACAGCGAGAAATGAGACTCTGCTGGTGGACCTG	
63	320	TTATCTCAAAAGACATATGCACTTG		P	CTAATAATAGTGTGTGATGTACCAATTT		GAGTCAGCGAGTGTCCCATCAAC	P	GAGTCAGCGAGTGTCCCATCAACATGGAATTTGTGATTTGTGTTA	
64	321	AGAACTACTGGATGATCTTTGG		P	GCAAGAGCGAAAGACANAAGC		TGATCNAAGAGAGTTCGACTCTGGGG	P	TGATCNAAGAGAGTTCGACTCTGGGGCAACCTCTCGGGTCCCTTTCCATTT	
65	322	CGGGGATCTCAAAAACCT		P	GGAACAGGATGATATTTGGG		TTTTTAAGTTTGTAGGAGCTGTTTGT	P	GGAGTTTAGAGGTCTCTTAATGATATGCAACAAACGCTCTACAAACTTTAAAAA	
66	323	GTGTTCCAGAGCTTTATACGATC		P	AGTTTGTGTTTCTGATTTGATAGTG		AGTGGGACTAATAATAACTTAACCTC	P	TCAATATGTGCGCACTTAAGAGAGSTTAAGTATATTAATGTCGCACT	
67	324	AAATCTTATCTCGCCCTCATG		P	CTTATGCTAGTTCCTCTCTAGTTTGA		ACTATAGTGAATTCGAGACTGATNT	P	ACTATAGTGAATTCGAGACTTCAATGTCTCCCTCTAGGTGGGAATGATATTA	
68	325	TTGTTGTTTACAAAGCATGAGC		P	ATTCTGATGCTGTTCTAGTTTGA		GGAGCATGAGGTTTCCAAAGCAGG	P	GGAGCATGAGGTTTCCAAAGCAGGCTCGGCGCCAGCAACAGAGAGGT	
69	326	GTTCGCTGTAGNATACACCCAC		P	AGACCTGGTTCATCTCATTTGATAGC		TAATTTAAAGGAAAGAGACTCAAC	P	TAATCAACGGGAAATTAATCAACCTTAGTTTAGTCTTTTCTTTTAAATTA	
70	327	CATATGCGTCAAGTGAAGTCAAG		P	CNAAGAGCTCAAGGAAATTTGG		CTCAAGAAATTCGAATGCGGATATTTG	P	CTCAAGAAATTCGAATGCGGATATTTGCGTTGGCTCAGATGAAATCTCAGAAATC	
71	328	CTGGATGTTTCAACAGCTGAGA		P	ACAGGAGCTCAAGGACACAG		CAGATATTTTGAGAGAGTAGGAAA	P	AAAGGCGAGTATCCATGAGGCTGAATTTTCCCTACTCTCTCAAAATATTCTG	
72	329	TGCTTCAAGCTCTGGCG		P	CAATAAGAAATTAACAACACTCAACTTAAAG		CAAGTGATCTCTTCTGCTCAAGCTC	P	CAAGTGATCTCTTCTGCTCAAGGACTCGGAAATGCGAGATGAG	
73	330	CTGCTCTTTTCTAATCAGTTTCTTC		P	ATTTGGGTTTGCATTAATCTCTCA		ATAATTGATATGAGATGAACACAA	P	ATAATTGATATGAGATGAACACANAGCANATAGANACTGATGTTCTGAG	
74	331	ATCTAATCTCTTACCAGTCTTCTCATGG		P	CTGTGATTTTGTGAAACACTTTG		GGCTCCCTCTGAGGAGTCTACCTAC	P	GGCTCCCTCTGAGGAGTCTACCTACCTGCTGAGGCGCAGCG	
75	332	TGCACTAAGTAGGCGTTGCGACA		P	AATAAAATTTTGGATGCGAACTTGG		AAAGGTGTTTCCACCATGATGATT	P	CAAGGCACTTTTACCCTTTAGATAGGATATCAATCAATGCTGGAACACCTTT	
76	333	CAAGTGCACAGGCCCA		P	GGCAATTGATGGGCGAGTG		XCTGACACCCAGACAGAGCAGCA	P	CCTAGCAGCCAGCAGGAGGACGACTCCCTCCCTCGCGAAGAAACGCGCTCG	
77	334	TTGGAAGTGAAGTCAAGCGCCA		P	CNACTATTTAAACAGTCCGCAAA		TTCAAGTTTCAAACTTTTCCGCACT	P	TTGGAAGCTTTGGAAGAGAACTCAAAAGTGGGAGGAAATTTGCAAGCTGAA	
78	335	ATCTCTGCACTCTGATGTTGATTT		P	GTGTGTGTGTGTGTGGCC		CTGTGATGATGATTTGATTGTTTGT	P	AGCATTAAGTAAATAGCCAAATATGCAAAACAAACTAAACATACATTTGACAG	
79	336	GTAATATGATATAGGACCCCACTAAGAT		P	GGAGAGTGTGAGGTTTGAATTTT		GGAGAGTGTGATGTTGATGATTGCT	P	GTCAAGAACTGATGTTGATGATTCTGCTTCAGACAGCTAAAACTAAATTTGAAA	
80	337	ACTCGGCTGGGTTGTTGAA		P	TAAAGGTAAATCCACAGACAC		TTGATGTTCCAGTGTGGGTGCATA	P	TTGATGTTCCAGTGTGGGTGCATAGAAATTTAGGTATTTTAGGCTAGTTTA	
81	338	CGGGGATCTGACTACTTGT		P	CATAACA TGCACTGAGTATATATATCTCC		TACAGTCCATGAGTGAATAATATG	P	ATACATATTTAGGAGACTTTTAAACAGCTATTAATTTTCACTGATGCACTGTA	
82	339	ATCTAGAAATPAGACAGCGCTGAATCTGTAA		P	GAATGGAGCTACTAGCCNCTACCC		ACTGGATCTCATCTTCTXAAAGCCT	P	CTCAGTTTGACACGACCTGCA TTAACGAGGCTCTGCTTCGCTACTTATTTGGAG	
83	340	AGTGAGTGAAGTGGCGCTACAG		P	CAATGGCACTATGGTGTGTATCG		ACTCGACTCGAGGTTCAATGAGGCC	P	AAACATTAAGCAGCAAAAGCTGTGTAGTCTTCGCTTCTGCTACTTATTTGGAG	
84	341	CGGGCATCTTCTTTAAACCAT		P	GTAANATGATATAGATGTACTATTTAAGGAGTGACT		CACTCCCGAGTTOAAGAGAT	P	TATCTATGATATTTTGGAGACAAAGCTGTGTAGTCTTCGCTTCTGCTACTTATTTGGAG	
85	342	CAACATGGTGAAACCCCATC		P	CACTCCCGAGTTOAAGAGAT		TTGGTTTGGGACTCCAGGA	P	AAATTAAGCTTTGGCGTAGTGTGGG	
86	343	TCACATAGAGCTACACATCAATTA		P	TTGGTTTGGGACTCCAGGA		TTGGTTTGGGACTCCAGGA	P	AACTTAAAGAAAGAGAAACAAAGAGGAGCTGTCTTCTTCGCTCAAGCAACCC	
87	344	TCCCATGCTCTGTCTTCC		P	CTTACCACCTTTCTCTCATTAAGCCA		CTTACCACCTTTCTCTCATTAAGCCA	P	GGGAGCCCTCGAAAAATGGAAGTCTTCTTCGAGGACAGCTTCCCATTAACAA	
88	345	CTGAATAGAGATTGGGGAAAGAGAA		P	TTATTAAGCTCTTCTCATATTTCTGGCTTT		TTATTAAGCTCTTCTCATATTTCTGGCTTT	P	GGCAATTTGGGGGTGCTCTGGAGAGGTGACATTTTAAACCAAGAGAGGCTGGCT	
89	346	CGCTCGGAGAGAGAGCAAC		P	TTTTCGCCAATCTCTATTTCAGTTAC		TCAGTTTGGGCTTTCTTCTTCTCA	P	GTAAATTTTGTGTGTAAGTATAGGAGAAATTAATCTACTTCAATCTGTA	
90	347	CGCTCGGAGAGAGAGCAAC		P	CAITTAATCTGCAATGACCAATGCC		AGCCTGTGCGAGCTCTMAAACTTGGCTTA	P	AGCCCAACATATCTGCGACCTTCTCTAGGCAAGTTTAAAGACTGGCAGCAGG	
91	348	AAACTGTCTTGGACTCAGGCA		P	TGTACTTAGCATACMAATCTCTAGGATGATT		AGCCTGCGCACTAGCTTTTTCG	P	AGCAGCCAAATATGTTTACTAGAGCTAAATTAATCTACTTCAATCTCAATCTGTA	
92	349	GAGCACACATGTTCTATTTGATG		P	AGCTCCGCACTAGCTTTTTCG		AGCTGTATATAGAGTGTCTCATAC	P	TAAGGTCTGTATTTTACAAAGTGTGCTATGACAGCATCTCTATTTATAGAGT	
93	350	CGRTATTTCTACAGGAAATAGTCTATTTA		P	TTTTTGCATGTGAACATTTAGTGTCTATA		GCTATAAATCTCTCTCTCAACAGT	P	CGAAATCTCTGGGACACAGCTAAGGCACTGTTCAGCAGAGAAATTTATAGC	
94	351	GAAACTATAGAGCAACAAACAAT		P	TTTTTGCATGTGAACATTTAGTGTCTATA		TGGAGATTAAGTCTTCACTGACTTC	P	ACATAGGTTAAGTTCATGCTTTTCAAGGGAGTCAAGTGAAGAGTTAATCTCCA	
95	352	GTGAAGATCGATACCTAAATATCTTATTAC		P	AGCTTTTGTCTCOMACTCGAGG		TKTTTCTCTTTTGTGTGTGTTTGC	P	CAAMAACAATCTGCGAAGAAATACCGATCAAAACCAAGAGAGGTAATCTGCA	
96	353	CGGGATCTCTACACAGAGAA		P	TGATTTTGAATGGAAATGCGATTG		CAATATGCTCTTTTTCACAAATGTT	P	AACATCCCATGCTCTATGAATTTGGAAGAGATTCATTTGTGAANAAGAGGAAATTA	
97	354	CTCTGAAGAAATACACATGATGAAC		P	GTATTCGATTTGCTTTGGCG		GAATTAAGCTTCTGAGTCTGAGCTTC	P	GAATTAAGCTTCTGAGTCTGAGCTTCGATTCGATTCGATTCGATTCGATTCG	
98	355	TCTGCTCACTGCATTTCTGGG		P	GATTCGAGCTCTGAGCTTC		GATTCGAGCTCTGAGCTTC	P	CGAAAAATTAATTTCAAGTGCATTCGATTCGATTCGATTCGATTCGATTCGATTCG	

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Row#	REF#	Upper PCR primer	1	2	3	4
			Modified	Lower PCR primer	Modified	Flanking sequence
197	461	AGCTGCTCTCTGCTGTC	P	GAATGATAGGATTAGTGTCTTTATAAGAGAGA	AGAGGGCTGGCACTTCTTTCTGTG	CACAGGCTTTTCTGAGTTCTCTGCGACAGAAAGTGCAGGCGCTCT
198	462	CTGGGGGCTTGTGTTT	P	AGTCGCCATATACCCATACATCTATAG	AGTTAAATGCCATTTCCGTCMAATTA	TTTTAAATTTGTGTAATAAGGTTGGTAATTTGATGGCAATGCAATTAAT
199	463	AGCTGTGATATATATGCGCTTT	P	CCGACGAATCTGAGAGCGCATG	CCAGCAAACTGAGAGAGAGGAGAGGT	CCAGCAAACTGAGAGAGAGAGGCGGAGCCAGCTTCTGTGACTG
200	464	GTTGAGTTGCAATATATTTCTTTTCCAC	P	TGGTAAATCAGAGACTGATTTATCATC	AAATGGGTCTAAATTTGCTAATAACTCC	AAATGGGTCTAAATTTGCTAATAACTCGTTTCCGAGCGGTTAAATATAGATCG
201	465	AGCTTTGGGTTCAAGAGGAG	P	TGGCGTAAGCCGCAAGCTC	TCGAAATGGCTCTCAGAGGCTTTTCT	TCGAGAGGGTAAAGTACTGCTGCTCCGAGAAAGGCTCTGACAGGCAATCC
202	466	TCCTGGGTTCCAGTACTGCTC	P	CACATATATGAGATCTCTATCTCTACAAA	AAAAAAGCCCAAAATTTAGCCAGCA	GCTGGGACTACAGGCTCGAACCAGCTGGCTGGCTGCTTAATTTTGGGTTTTTT
203	468	ATAGAAATATATAGCATGAGGAATTC	P	AGCTAAATTTAGCATAGTACTGTGAGA	GAGATGTGTTAATAAAGAAACCA	TTTTGCTTCAAAATGTTATTCAAAATGTGTTTCTCTTAATAACCAATCTC
204	469	CACCTTTACTTAGTATTCAAAACCTCTGCA	P	GTTTATACTATATGCAATATATTCCTCATC	ATTGXAAGAAATGCAATCTCTCACTAGG	TTTCAGAAATCAATCTCTCACTAGCAAGTTGCTATATAGCAATCAGGAATAT
205	470	TTTTTTTGGTCATATAAGCTTAATGGG	P	GTACTTTTAAATGAAGGCTGTGGACT	TGTGACTCCCATGAACTAGATATG	ATTTTTCAATCTCAGATCAGACAGCAATCTAGGTTTCAATGGGTTCCACA
206	471	CAGCTCTCCAAAAGCAAAAGTC	P	TCTGTAGCCAGAGGACTGTAT	ATTAAAGAAAAAAGAAAGATAG	AAAAATGCTTACCCAAATCAATAGCACTGATCTTTTTTTTTTTTCTTAAT
207	472	AAAAAATAGCTGGCATCCAA	P	GCGAACTCGTCTGAGCTC	GCTGACCCAGGCGCCCTTGTCTCCGCTGGGTCCGAGGAGCAGCAGCT	GCTGACCCAGGCGCCCTTGTCTCCGCTGGGTCCGAGGAGCAGCAGCT
208	473	ATTTCGTGCTATTCAGAAATGCTCTTT	P	GATTTAAATGCAACCAATCAGC	TAAAAAGCAAACTTGTAGCAATCC	ATCTTCTGTGATCTGCTACTTTTGGATTTGCTGCTGCTGCTGCTTTTA
209	474	TTAGTGCAGTAAATATAGGTGCTAAGA	P	TTCTAAATGCTTCACATCCGC	CATTACATTTTCTGTAACTTAGCAGA	TTTACTTTTTCTGTAACTTAGGAGATAGGTCACCCATCAGCAAAAGGTAA
210	475	GAGCGAGTATAGGTTTATTTTATTTCA	P	TATGGCGCTTATTTCTGATATTTCA	TAATTAATTTTCTGTCAGGCAATTT	TAATTAATTTTCTGTCAGGGAATTCGAAACATTAATTTGCGMACTGAAATAT
211	477	GACAAAGTTTGGCTGTGATCTTC	P	CCAAATCTGCTCAAAATAGGCA	CAAGATACAAAATTTTTCAGACAATG	AAATCAATCTAACTCTGGCAATTTTCAAGATCTGCTGCAAAATCTGATCTTTG
212	478	TTAGTTCACAGTACTGATAACCCCTTG	P	GCCCTTCCACTCTACACACAC	CACCGTGGTAGCTTCTCTCATGCGCT	GCACTGGTTGMAATACTAATTAACAGGCAATGAGGAGCTGACCCAGCGT
213	479	TCGACAGAAATAATGCAATCA	P	GTTTCTAATACTGATTTCTTTTCCAGTG	CGTTCGAACTACTGAACTCATTAGT	CGTTGAACTACTGAACTCATATAGTAGCCGAGCAATAGACTGAGTCGAAA
214	481	TTTTTTTTTTTAAAGCAACTCTGCC	P	ATTGCTTAAACCCAGGAG	GGGAGGTGCGGTGATGACTATAGATGAC	TCCCGCGAGGCTGGAGTGCCTGGGTGGGTGCTTAATCTTAGCTCTACTGCAAGCTCC
215	482	TTTAGCACTCAGAAAGGAACAAG	P	CTATAATGCAACTCTGTGTTCT	CTTAACTCTGGTTCCTGTTTTTT	TCGCTCTCTCTGTTTTTTTTTGGTTTTTGGTTTTTAACTGAGTCAATG
216	483	CTAAGCAAGCAATAGAGAAAT	P	TGCATAAGGTGMACTCTTCTGGC	AACATTTGGAAATTTTCTTACCTTTA	AACATTTGGAAATTTTCTTACTTTACGACACTTTTACTGCTCTCAGAGAA
217	484	AAAAATAGCCTTCACTGCAAACTATGTATA	P	CAGGCTCTTGACAGCTCTTCTC	CTTCTCAAGGAACACTCTGXTCTGGCT	TGGCAATGGAATCTGAGCAAGCCGAGCGAGATCAGAGATTCCTTGGAGA
218	485	TTATCTTAGGTTTCCGAGGCCA	P	CATATGAGTCTCTCAATTAATGTAGCTC	GCGTCCCTCTCTCTAAATCCGAA	CAGCGCTTCAAGAGCTCTGATGCGGTGGGTGGAATTTAAGAGAGAGGAGCC
219	486	AACCAAGTATGAGAGCCTTCTCC	P	CACCTAGAGGTTTACAGAGACCC	AGATTTCTCAGGAGGTTGCGAGAAAG	AGATTTCTCAGGAGGTTGCGAAAGGTTGACGCGCTCGCATGAAAGGCTC
220	488	CNACTCCACTTCCGAGCT	P	CTACTAAAAATACAGAAATTAGCGAGC	TTCAAGXAAATTTCTCGTCCCTCAGCTC	CAGCAAAATTTCTCGTCCCTCAGCGCTCCAGATAGCTGGAAATACAGGCGCAT
221	489	GCTTTTCAGAGGCAATCAAGC	P	TGCTTCAAGCGCTGATGATTTCT	CAATTTTAAATCAGAGGATTT	GCTTTTTTTTTTCTGACCAATCTAANAATCACTGTGATTAATTTAATTTTG
222	490	ACAGCGTCAAGAGACAGAC	P	CTTAATCAGCGCTCTTGATATG	CAATTTTGGTAACTCCXAGTCTGCT	TTCTTTGGTAACTGACTGCTGCTAATAACGAGGCTGCAAGCGCTCGAAG
223	491	TTATATCTAGTATGTGTAATTTTTCAGGGT	P	CTAATCTTTACTGTGAGTCTTAGGAAGGTAAATTA	TCGAGAGAGCAAGCCACTTTAGCTAGCA	TCGAGAGCAAGCCACTTTAGCTAGCAAGCCCTGGGGTTGTTCACACAAG
224	492	CACCTTTATCTCAGAGCCACT	P	AGTGCATTTTCAATTTCTGCTGTATG	TTCAAGGCTTTCTGGCAATCTTTCTTGGGA	CAAGGCTTTCTGGCAATCTTTTTCGAGAACTAATAAATACATAATTTTATA
225	493	AAATATCAAGCCTTCTTCTCATG	P	GTTATACTACTACTTTTAGACAAAGATCGAGC	GACCAACACTCAAAAAACAAT	ATTAACAATGTCTGCTTTGTAAGAGATTTGTGTTTTTTTGGAGTTGGTGTGTC
226	494	ATAAGATGAAGTGCAGATGCAATG	P	CTTTCAATTTCTCTTCTCTCTGCTA	TTTGTAAAAACCACTGTGCGCATAGT	AGACAAGTGTGTTGTGGCACTTAGTCACTATGCGCAAGCTGTGTTTACAAA
227	495	CATGCTATCAACTCTATGTGCAT	P	CNAGTGTGTAAAGCAAAAGCAG	GGGAGGGAAATAGCAAGTGTATA	GGCGCGAGCTCATTTGATTCGTTGTAATACACTGCTGCTATTTCCCTGCC
228	497	TTGCAGTGAGCGAGAGT	P	CATATACCTGTTGGGAGATG	ATCGGGAACAGATCGAANAATGATGA	AAAAAAAAGAGAACTATTTCTCTCACTCATCATTTTTCGCTCATCTGATTCGCC
229	498	GACACAAATTTGCCATGCAIT	P	GGCATTTGGCTTAATGACCAIT	AGTGAANAATACGAGGAGAAAG	TTACATGCATTACAAAAATAGCAATGCTTTTGGCTCTCTCATATTTTCACT
230	500	TATGTGCTTAAGTTAAACTGAGGGTCC	P	CATCATGCTCTGGGTCACATTA	TACAGCAACTACCAATTTTCACTGTC	CTGTTTACAAAATGACACATTTTCAATGCAAGATGCAATTTTGTAGTGTCTGTA
231	501	TTTAAGTTCTGGGATACATGTGCTG	P	AAACCTAGATGATGAGTTGATGG	TGCAGGTTTGTACATAGGTATACA	TGCAGGTTTGTACATAGGTATACAGTGCCATGCTGGTGTGTCGACCCCA
232	502	CTAAATACCAATTTGAATGTAAGAATGTA	P	AGTATAGTGTGATATTTGATTAAGTATACATTA	CTCAACTCAACTCTGCTATATATGTA	CTCAACTCAACTCTGCTATATATGTAATAGATGCATATCCCTCATAGTGTAGTGT
233	503	TAAAGTCGAGTAAAGTGCGTTA	P	AGTTTCAGTTCTACTATAAAATCTGGGCA	GGCAGGACTCTGAGCAAACTCCC	AGGAAGTAGTTCGAAATTTGTGTTTGGGGGAGTTTGTCTCAGACTGCTGTC
234	504	TGAAGGCGTTTAGTGAOCMAATTA	P	GGGAGGTAGAAATTTGAGAAATAGGG	AGGGGATXGCTAAATTTTCTXCAATTCG	ACTTGGTTTCAGAGCTCTTCGATTTTCCGAATTTGCGAANAATAGCAAAATGCC
235	505	GCACCTTAATCTCCATCTGAGG	P	TCTAGATATTTATGTCGAATCCCATCA	TTTTTATAGCATAGTAAATTTTGGCAATTT	TTATAGCATAGTAAATTTGGAACAATCTGTGTGGCATTTTATATGCAAAAAC
236	506	TGTAGAGAGTATGTTATGCGAGC	P	AGTCTCTTGTGACAAAATGTATGACAG	GACCGCAAACTGATATTAATGCTTTCA	CCGAGAAACTGATATGAAATCTCGCAATTAATTTGCGAAGCAAGCAAGCTGTC
237	507	TAAAGATGTCCTTAATCCAGTTTTCGC	P	AGCACAGAGCGCAAGTTGGGA	GACGAGGCTCCAGCAAGCAAGCGCA	GTAATCTCTCACAGATTAATCCAGCGCTCCCTTCTCTCTGCGAGCCCTGCTC
238	508	GGCTTTTCCGACACGNC	P	CTTCGCACTCTCCCTCTGCTCA	GAAGGGTAGCCGAGGCTAGAAATTT	GAAGGGTAGCCGAGGCTAGAAATTTCAATTCAAACCCTAGGCTTCCCACTGTA
239	509	GTGAGGCGCCGACATGAG	P	CTGCTCCAGCTTAATAACCCAAAC	GGCTCCGCACTGCAATTTCTGACCA	GGCTCCGCACTGCAATTTCTGACCAACATTTGTGTGGCAATTTATATCTCTACCA
240	510	CCAGGAGCTCMAAAGCACTG	P	ACCCCTTTTCTTAGCGATGTTT	TGMAAATAAATAATTAAGAANAATA	AACATAAATAAATATGACTTAATAGTATATTTCTTATATTTATTTTTCGA
242	511	CCACTGACGCTTAATCCAAATGTACAC	P	TCTTTTCAATCAAAAGAGAAAGACAGC	ACAGATGTCAAAAATAGCAACAACACTCTTAACAGAGCTGTGTTCT	ACAGATGTCAAAAATAGCAACAACACTCTTAACAGAGCTGTGTTCT
243	512	TGCTTCATATATATGCGGGA	P	CATGTCGCGCAAAACCCCTCT	AGGGCACTTGTGTGGTGGGAATAGGC	AGGGCACTTGTGTGGTGGGAATAGCGGGAATGGGGGTGGTGCATTTTATGCA
244	513	ACATACAAATTTGATGCGAATATATGACAA	P	TGCAATGTAGAAATCTCCCTCTGCGGC	GNACTGTTCGAAATCTCCCTCTCCCA	TGCAAGAGAGTTTTCATTTTAATTTGTCTGGGGCAATGGGGGCAATTTTCAAGGT
245	515	TTTACATGCTCAGTAGCCTCAAGC	P	TAAATGACCACTGGGCGCTG	CCTGCTCTCTCTGAACTCTCTCT	TTCCGACAGAGGGGCAAGTTCACACAGAGAGGATTTTCACAGAGGAGG
245	516	TTTATAGGGTTTGGTTTTTATGCGGA	P	GAAGCTGCTGACTGCGCTGA	TGATCTGTGTGAAACTTAGTGTAA	TTGAGCTGTATAAAAACCTGTCAAAACACTTACACTTAGTTTTCAACAGAGATCT

[illegible]

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	3	Modified	Flanking sequence
295	581	GCATTGCGAAGCAAGTTGAGT	P		TTAGGCGATTGCTTTTGTGATG	TTGATGTTXCGAAGACGTGGATTCAG		CTTTTAAATCTCTACTGCGGCAACCGAGCTGAAATCCACTGTTTCTGGAAACATC
296	582	CATGCGAGTGGGACCATG	P		CTCTTCTGCTCCTGCTGTCTT	CTCTCCCACTTTCCACACTCCCATCTA		GACTCGAAGTGGCCCATCCAGACTAAATAGATGGGAGTGTGAGTGTGAGTGGGAGT
297	583	TGTACAGCACTGGCAGGAAA	P		CACATCGACTGTATGATCTGCC	TTGAGATATCTCTCGAAACTACTCT		TGTGTTCTCAAAAACCTCTCATTTAAAAAGAGTGTGTTTGGAGGATATCTGAA
298	584	CGCTACTATTTATTTCTATCCAAAGC	P		CGCCAGCAGCAGCTTCC	TTTGCCCTCCCAACTTTCCAGCTCTG		CAGCGCGCGCATCTCGACACTCGCGCGAGCTGAGTGTGAGTGTGAGGCGGCAAA
299	585	CGACAGAGTATATCAAGAGCGCAAG	P		TATGCTCAGAGTTTTGTGACTGCAATT	P		ATAATTTACCTCGCAATGCAAAATTTTGSTCNAATTAGGCAAAATTCGAATTGCAAT
300	586	ATCAAGAGTTCAGGAGTTTGTAG	P		GAITTAGCAGCACTGCCAACC	GAATTCGATTTTGTATTTTGTAGTAGAGA		GGCCTGCGCAGATGTGCAAAACCCCGTCTCTACTATAAAAATACAAAAGTTTAG
301	587	AGATGGTCAAAACCCGCTCTAT	P		GAATTCCTGCTCAGCTTCC	TGCTBTAGCTGGGATTACAGCA		AAAAGTTAGCGCGGCGTGGCGAGCTGCTGTAATCCGAGCTACTYAGGA
302	589	TGCATATCGGACAGCAAGCTATATC	P		CAATCTGAATTTCACTGAAAGTTCTTAG	CAGAGCTTGAAGTAAAGACTTGGCCCTA		CAGGCTCCCATATGTGAAATACCTAACTAGAGGCAAGTCTTGTAGTCCGAAGCTC
303	590	TGCTGTGCTGCTTCCCTC	P		CATTTCTTACCAATTTCCCAAT	ATAGGATTTACTCACTCATTTCAAGCA		ATAGGATTTACTCACTCATTCAGCCCATCTCATTCATTTGGCCAGATCTGTAGTT
304	591	CAACACACTTTATCTTCTTTGAT	P		TCMAATCATGTCGCAAAAATATCATG	TCATGTAGATATCAAGTGAAGTATA		TGATCTCTGGGATCTTAACCTGCTGATATACCTCACTTCAATCTTCAACATGA
305	592	TCAGCTGNACTCTGCTCT	P		CMAAATAGCCAGCATGTGGTG	CAATTTCTTGCTGCACTGAGTCTCCAA		CAATTTCTTGCTGCTCAGTCTCCGAGTGTGAGATCAGAGCAAGCCGCTGA
306	593	GGTTTCAGACGATTTCTTTGCG	P		CATGCGAAAACCCGCTCT	GGATCAGAGGACCCGCTCACCACAC		GGATCAGAGGACCCGCTCACCACAGCTGGCTAGTTTTTGTGATTTTTTGTAGTAG
307	594	CGATCTTTTACCAACATGCTTCTT	P		TGTCAGATCATGGCTATGTTCAATT	TTGTTTGCAGCTTAGXAAATCATTTTACC		AACATGATGTTTTTAAGACATATACCGTFAAAATGATTAATCTACAGTCCGAAC
308	595	CTTGGAAGCGGATATATGGAGCG	P		TCGTGTGTGTGTGCTGCTGT	TCATTTCACTATGTTGCTGCTCTCT		GTATCTCAACTTCACTATAAATATATGAGAGAGCAACATTAAGTCAAAATGA
309	596	TAGATATCTATTAATCTTATTTCTGATGATTC	P		CAITTCAGGCTCAGTGCAGCTGTG	TAGTAGGCGACATATCAAGCACTTTCTAT		GTAGGCGACACATTAAGCATTTCAATGTGCATTTTTTTTCTTTTCTTTTCAGAC
310	598	TTCCCAACTACTATACCGAGC	P		CTTGCCCTCCCAAGTGT	GAITTTTTTATGTTCTTAGATGAGGC		ACAAATAGAGAAACCCAGGCAATTCGACGCTCATCTAGACATTAANAATC
311	599	CGCCCTGTAGTCCAGCTA	P		TTATTTTATTTTAGACACAGAGTGGCT	TTGGAGGCTGAGGCGAGGATGGGTTGACCGCGAGGCGAGGCTGCGA		TTGGAGGCTGAGGCGAGGATGGGTTGACCGCGAGGCGAGGCGGCTGCGA
312	600	CATGCATACTCTGACAGAGA	P		GGGACCCCAAAAGCTGA	TAATATATAGAGGCGCACTCAAAAT		TAATATATAGAGGCGCACTCAAAATAGGCGACAGATGTTTGGTTTGGCCTTA
313	601	GTGTTTAAATAGCTTTCTTCTATCATATAA	P		TGCCACAGAGACAGTCTCTTTTTT	TATGACAGAGTCTTCTGGAAGATTTCCG		TTTGAATGCTTCTAGCTCAGTTCAGTGGCGAAATTTCTTCAGACACTCTGCATAT
314	602	TAAATGGCTGTCTTATGATATCCA	P		CTTTTATATCTCCACTGCGACGG	TCATCATTTGAATTTAGMCTTCTGGCT		GAITTAAGCGCTTGAGCCACCGCGCAAGCCAGACGAGAGCTCTAAATTTCAATGAT
315	603	TGGCGTTCCAGAGTCT	P		GAAATTTTATATCAGCAGCATGTC	GTGTGCAAGTTTAACTCTTCTGCTCT		GTTTCAAGTTTTAACTCTTCTCTCTGCAAACTCCCTGAGCGAGACTCTTTT
316	604	CTTGCACTTTCTGCTTTTCTACT	P		GAACACATCATGCCAAAACC	CATGAGCAGATCTCGAGCAGAGA		AAITTTCAATGATGATGTGCTTTTTTATCTTTTCTCTCTCAGCATCTGCGCTCATG
317	605	ATTACAGCGGTGAGCGAACC	P		GTGTTTCTCAGAGCCGATTC	ACTTCATCATACAGTCTTTCAACAA		ACTTCATCATACAGTCTTCAGCAACCTTGAAGAATTTGCAAGCTTCAGC
318	606	TAGTGTGCAATTCGAGTTTCTGCT	P		ATTTCTTGAAGCGAGAGATTAAG	ACAGACCCCATCTCTCAAAAAAA		CTACACACCGCACTATTTAAAAAAATTTTTTTGTAGAGATGGGCTCTGCT
319	607	AAGTAACTCTGCTGCTGAGACTC	P		TAGCATAAATTTTGAAGACATATGG	CACATGTGCTAGTTTATTTGCTCTTA		ACATGGAATAGAAATATCACTTAACTTAACTGAAAGAATAAAATACCAATGTG
320	608	CGACGATTTTCTTAAAGAGCTAAACA	P		CAGTCTCTGCTGTGAGAGG	GAGAGGATGGXCTGGXCTGGTGCCAC		TCCTAGATCCCTCAGCAGCTCTGTGTGGTGGCAAGCCGAGCCGACCTCTC
321	609	CGTGTACGAGAGCTGTTTT	P		CAGACAGGACGAGCGGGC	CGACTGGCAATTTGGCAGTTGCAAGGG		CGACTGGCAATTTGGCAGTTGCAAGGGCTCTGAGCGAGGCGGCGATCTC
322	610	CAGGAGCTGGGCAAAAGTG	P		CACATGAGGAGCGAGCTCTCATACAC	TATCATACACATCTXCTGCGCATTCAA		ATACGACTTGGCGTGCACTTTTGGCGAGTTGAAATGGAGGAGATGTTGATGA
323	611	TTTTTTTATATTGGAGTACTGCTCAACA	P		GTTTCTGGCGAATTTTCCAAA	ATAGACAGTACGACCGCAAGACAA		CACCTCTGAGTGGCTCATTTTTCGAGCTTGTGCTTTTGGCTGTGCTAGTTCTCTAT
324	614	AAATGTGTATTAACACATCTCTCT	P		GTTTCTATCTTCATTTGTTTCTATACCTTT	CAATATGATAGATGGXAAAGATTTCTAT		AAGAAATTTGGCTGGAGCAATCTCAGATAGATCTTCTTCCCATCTTACATAT
325	615	TAGGAGACCACTTACTACTATAGATCAT	P		GGAGACCTGCTTGAACCCAGA	CGAAGCTCAGAGTTXGAGTAGGCCA		CAGGCTGGAGTGGAGTTCGATTCGATCTCATCATCTCAGTCCGACTCTGCTCTCA
326	616	TTTTTTTCTTTGTTTTCAGATGGAGT	P		ACTTGACAGCGCGGATTCG	TCGAAGGAGCATCTCTGGATATCA		TCATCAGGACGCTATTCTTCTATATGCTATCATATCCAGAGATGCTCTTCTTCA
327	617	TTGACATATTTTCCCATCA	P		CGCATTTGCACTTCCACCC	CAITTTTTTTTTTTTTTTTTTTTTTTTTT	P	CAITTTTTTTTTTTTTTTTTTTTTTTTTTTTGTAGATATGGAGTTTCACTTTTGTGTC
328	618	GAAGTCAGGCTGAGGGACA	P		GTTTTAGAACGCTTAAATATGTC	GAGACAGGTCGACAGCGAGCCAGGGA	P	ACCGTTTTGTCATAGCTGCGCCCTTGGCTCGGCTGCGCTGCTGTCACCTCTC
329	619	TTGCTTTTATCATGCTCTCATCA	P		CMAAGCTCGGATTTTACAGTC	GAGTGGCTCAATTTCTTTTGGCCACAA	P	GAGAGACCTCTCGGATTCMAAAATAGTTGTGTCGCAAAAAGATATGGCGAGCC
330	620	ACTAAAAATACAACTGTAAACATCATGG	P		GMAAGCTGTCGCCATTTGAT	TATTCMAAAGCTAAATGTGGGTCT	P	TAITTCMAAAGCTAAATGTGGGTCTCTGCAAAATGTCTTCCCATCTCAAAAAGT
331	621	TATAAATGACAGCAAGTCTTACTTTGTAG	P		ACCTTAAATTTCTCTCTTAAAGGCC	GGCCCTATCTCCAAAATGTAGTCACA	P	TTATA GTTGCMAAGTCTAGCTTCCAGTGTGACTACATATTTGGAGATAGGGCC
332	622	CTGTTTAGGAAAAGTTTCACTCTCTCT	P			TTGCTGTGAGTGGAGCGCAACTG	P	TTGCTGTGAGTGGAGCGAGTGGCTGCTCATCTCTCAGCTGCACTGCG
333	623	TTTCCCTTTCATTTCTTTTGTAGAT	P		GGCCTGTAGGCTGAGGTTGCT	CTTXGCTGAGCGAXCTGCTAGGGCAC	P	TOCCTGAGCTGCTTACTAGTGGCGAGCGAGTTCGCTCATCTCTCAGCTGCACTGCA
334	624	TCGACAGCCCATCTCCCC	P		GAITTTCTCCCTTACGACACCC	CMAGTGCGAGGCACAGTGTGAACACCGAANAATTTGCTGCACAATTTGTAGGTC	P	AGTGTGCGCACAGTGTGTGAACACCGAANAATTTGCTGCACAATTTGTAGGTC
335	625	TAAAAAGATGCTCTTCAATACACCT	P		CTCTTAAATAGCAAAAATAAAATATGAGCTATAGG	GACCAATAAGACGACAGAGXCCAGACATA	P	TTAAGAGCGACAGCGAGCGGCTCGCAGTATGTCGTGGCCCTCTTGGCTTTATTTGG
336	626	ACATAGTAAATGCTCAGTAAATGTAGCA	P		GTACCTTTCAATGCTGGCC	GGXCCAGANAATGAGGCAAGAGGGG	P	GGGATTTCAAGATAGGAGCGGCCCCCTCTTGGCTCTTGGCTCTTCTTTGTGG
337	627	AGATCTCAGGAGAGGGGCTG	P		GAAITTTCTGGAGACAGCTGCG	TGGGMAAATAATACATATAGTGGATACATCTGAGAGGCTTTTCTGGAAGAATAGTGTCTCTGG	P	AGCTATGACTCTGCTCTTCTGAGAGGCTTTTCTGGAAGGCTTTTCTGGAAGAATAGTGTCTCTGG
338	628	AGACAGATAAAGCTAGTCTGCTGTCAGG	P		CAGGTTCCGCCACCTTGT	CTGTCACTCTCTCTCTTTTCCCCC	P	ACTAAACATGGGGGTGAGGTTGGAGCGGCGGCGGCAACAGACAGTGCAGCA
339	629	ACCAACTAGTGCAGAGCC	P		CTTATAGTTTCTTCTCTTAAAGGCC	GGCCCTATCTCCAAAATGTAGTCACA	P	GGCCCTATCTCCAAAATGTAGTCACTAGCT

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	Modified GBA primer	3	Flanking sequence
344	634	AGGACTAAGCCCAATTTGG			CTTGAAGTATGCTGAGTGGAAATTT		P	CCCCAGTCAATGGGGTGAATTTGCTTTT	GTAGCCCAAGGAATGAATGGTGT
345	635	AACACAGACAGCCCAAGCC		P	CAACCCCTTTCTGATTTCCG		P	CACAGCACTCGGGTCTCTTCACTC	GCAGCTGCTCAGGAGCCAGGAGTGAAGGAGCCAGGCTGGCTG
346	636	TGACAGTCCGCGCCAGG		P	GGCAGTTGTTGAAGCTGGG		P	CGGGTGTGTGCTGAGTCAAGCTGGGGT	CACACTCCAGCTGGGATTTGGACAGAGCCAGGCTGTGAGTGAACAGCC
347	637	CAACAGACATTCGATTTGCA		P	TGGCTAGTGAATTAATCTCCTAT		P	TCAGCTAGTTAAATTAATCTCCTAT	TTGCTTTGGTTTCAAGCTATTAAGATAGGAGATTAATTAACATACAGTCA
348	638	TGACCCCTACAGACACTAGTGTG		P	GTATGTGCTGCTAGCTAGATTT		P	ATTTCAGCTATCAATCCCTATGTC	AGAGGGAGTCTCTAGAGTGGAAAGACATAGGATTTAGTCTTAAT
349	639	GGCTCCACTTTTGGAGTCACT		P	AGAGCTGCTTTTAAATTTCCAC		P	ATGTGAGAGATTTCTTAATTTACAGCTATGAAATTAAGCTCCATCTTTCTG	GTAGAGAGATTTCTTAATTTACAGCTATGAAATTAAGCTCCATCTTTCTG
350	641	TTTAAAGTGGCCCTGCTGTATC		P	TAATAGAGTGGTGGTGGTGGT		P	CAAGCGTATTTAGTCTGAGTGAACCA	AGGGTTTTAGTCTGAGTGAACAGCTATGCTGCTATGCTCTTCTTCA
351	643	TTAGGTGTACAGATGATCTGAGATG		P	TTATAGATGCTCAAGTCTCTGATATG		P	TTAAAGTATGTGGAGAGATTCGGGTGTTTATATGCAATTAAGCTCTGCTTCA	TTAAAGTATGTGGAGAGATTCGGGTGTTTATATGCAATTAAGCTCTGCTTCA
352	644	TTTTTTTTTTTTCAGACAGAGAGT		P	CGAGGAGATCACTTGAACCC		P	AXCCGGAGGAGAGGTTXAGTGAAG	CTGTGACCGGGCTGGTGAATCTCGGCTCACTGCTGCAACCTCTGCTCTGGG
353	645	ATCCGCTCTCTGTCAACC		P	GATACAGGAGAGATGAGG		P	GGCAGAGGTGGAGGTTGTAGTGA	CTGAGTGTGAGTGGATTAATCAAGGCTCACTAGACCTCTCACTCTGGG
354	646	TTGGGGGAGGAGCCAT		P	CTCAATGGAAAGTCCGTTATG		P	AGGATTTAGTGAATGCTGCTGCTG	GAGACCTGCTCTGAGGATTTGACAGGAGGATCTCTCACTTAATCTCT
355	647	TTGGGGGAGGAGCCAT		P	CTCAATGGAAAGTCCGTTATG		P	TTAAATTTAGTGAATGCTGCTGCTG	GAGTCTGAAATTTATTTCAACATATATACATATATACGTTAGGATTTTAA
356	648	CCACTGGAAGGTGAGCC		P	CAATTAAGCTGGAGAGCC		P	CAAGAGCTGGGGCCCAACAGCAGCA	CAAGAGCTGGGGCCCAACAGCAGCAGGAGGAGTCAAGGCTGTGTGT
357	650	AGTCTCTCCCTTGGGTCTT		P	GGTGTGAGAGGAGGTGATTT		P	CACTGAGCACTGATTTCCCAACAC	CACTGAGCACTGATTTCCCAACACCCAGGAGGATTTCAAGGCTGTGTGT
358	651	TTGTGTGTGAGACACTGAGC		P	GCTGAAGGAGGAGGTGATTT		P	ACAGGCACTGATCAATGTGAGCC	CCCAACCTGCTCTGAGGATGAGGCTGACATTTGATGTGTGTGTGT
359	652	TGCTCCAGAAATCCAAACATTT		P	TGAGCATCCCAATCCAA		P	TCGAAATTTAGAGCTGCTCAATGA	ACCAAAATGATTTTCAAGAGAGTGTCTATTTGAGGAGTCTTAAATTTTCA
360	653	GTCCAAATTAATCTAAGTATGCACTT		P	AGGACCTAAGTGGTGTCTGACAG		P	ACAAGCAATGCACTTTCAATATAAT	CCCTCTGCTTTTCAATCACTGCTCACTATATTTCAAGGCTCAATTTCTGT
361	936	CTCATTAAGCAAGATAGAGAAATTAAT		P	TGACTGTGAGTGGCTCTTAATTAAGAT		P	TGCTGCAATTTGGGCAACAGAGAA	GTGCAATTTGGGCAACAGAGAAATTTCAAGGCTCAATTTCTGT
362	937	TGATTTTTCATACAGCTCAATATAC		P	AAATTAAGCTGAGTGTGCTTAAGCTT		P	TTAAGACCAATTTCTCTGAGGCAAG	CCATTAATTCAGAGCTGAAACAGATGCTTTGCTGAGAGAAATTTGCTTT
363	938	TCAGTGTGCAATTTTGTGTGA		P	CAATAGCATCTATGATCAGCC		P	GAATTCCTAGGAGGAGGAGGAGG	GAATTCCTAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
364	939	TCTGAATTTTTCGACATCACTG		P	GTAATCTGATTAATTTCTGACCA		P	GTCTTAATGAGTTTCTGCTGAG	GTCTTAATGAGTTTCTGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGG
365	940	GCAACAGGCTGTGTATTAAT		P	TCCCATCTGAGTGAAGG		P	AAGTGTGATTAAGATTTAATGAAG	TTTATGCAAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
366	941	TCAAAACCAAGCATTTGAGG		P	GCAGGAGGAGGAGG		P	TGCAATTTGATTTTCTGAGG	TGCAATTTGATTTTCTGAGGAGGAGGAGGAGGAGGAGGAGGAGG
367	942	TAGCTGGATTTAGAGG		P	GATCATTTGAGGAGGAGG		P	AAATTTTGTATTTTCTGAGG	AAATTTTGTATTTTCTGAGGAGGAGGAGGAGGAGGAGGAGGAGG
368	943	ATTAGAGGATTTAGAGG		P	GTCACAGTGTGAGGAGGAGG		P	TCGTGGAAGAGGAGGAGGAGG	TCGTGGAAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
369	944	TCTGGATTTAGAGG		P	AACTTTGGCTAAGGAGG		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
370	945	CTCAGATTTAGAGGAGGAGG		P	GTAAATTTGCTGAGTCAAGTGA		P	AAAGCAATTAAGCAAGGAGGAGG	AAAGCAATTAAGCAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
371	946	ATCAGAGGAGGAGGAGG		P	CACAGGAGGAGGAGGAGG		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
372	947	CTGCAATTTGCTGAGGAGG		P	TGGCTAGTGTGCTTTTACAGTGA		P	AAAGATTTTGAAGGAGGAGGAGG	AAAGATTTTGAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
373	948	ATATGAGAGGAGGAGGAGG		P	AGTAAAGAGGAGGAGGAGGAGG		P	CTTTAGGATTTGAGGAGGAGGAGG	CTTTAGGATTTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
374	949	TCATGTTTACAGGAGGAGG		P	TAGAGAGATGAGGAGGAGGAGG		P	AAAGATTTTGAAGGAGGAGGAGG	AAAGATTTTGAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
375	950	AGGAGGAGGAGGAGGAGG		P	AAATAGTCAATCTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
376	951	AAAGAGGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	AAAGATTTTGAAGGAGGAGGAGG	AAAGATTTTGAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
377	952	ATGCAAGGAGGAGGAGG		P	AAATAGTCAATCTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
378	953	AGGAGGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	AAAGATTTTGAAGGAGGAGGAGG	AAAGATTTTGAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
379	954	GGCTTTTGTGAGGAGGAGG		P	TTGTTTGAAGTATATGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
380	955	GTCTATATAGGAGGAGGAGG		P	AGGTTAGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
381	956	GGCTTTTGTGAGGAGGAGG		P	GGCAATGTTGAGGAGG		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
382	957	CTTAGAGGAGGAGGAGGAGG		P	GGCAGGAGGAGGAGGAGG		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
383	958	AGGAGGAGGAGGAGGAGG		P	GATCCATTTTATGAGGAGGAGG		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
384	959	ATATAGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
385	960	GTACAGGAGGAGGAGGAGG		P	TTGTTTGAAGTATATGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
386	961	GAAAGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
387	962	CTTAGAGGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
388	963	ATATAGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
389	964	GTACAGGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
390	965	GTACAGGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
391	966	GAGCTTTAGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
392	967	ATTAAAGGAGGAGGAGGAGG		P	GGTGGATTTGAGTCAAGTGA		P	CTCATTTACCAAGAGGATTAAG	GCACATCTCCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG

[illegible]

[illegible]

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	3	Modified CBA primer	Flanking sequence
491	1078	GTTCCTGMACTTAATGATTTTAAAA		P	ACGATAAGCATGGCATAGATTC		GATTOMAGATTTTTTTTTTCTGTAA		TTAGTTCTTCTTGATATTTACAGCTTACAGAAAAAANAATCTCTGANTC
492	1079	TGTTGATCACTGCTGCAAT		P	CTGACTTAATTTTGTATTTTGTAGTAAATGG		TGCAACTCTGCACTCAAGTCAITTC		CAACACTTTTGGGAGGCGCTAGCTGGCCCAATCACTCTCAGCTCAGAGATTCGA
493	1080	AAAAACAACGACGCTCCACA		P	AAAGTATAGGAACACTCAGTTTCG		GAACTTCTGAGATCAACACCCAC		TTCCGATCCTCTTGACAGCTCTGTGGTGTGTGTGATCTGCATGTTTC
494	1081	CTGCTAGAAACTCTGTAAATGTTCT			AGTTCTTAATTTCCACTCAGACC		GATCAAAATGCTATAGTATGCAATGAAGTTTCAGATTCAGATGAGCTCTC	P	TTAAAAATGCTATAGTATGCAATGAAGTTTCAGATTCAGATGAGCTCTC
495	1082	TGAATACACACACAAATGAC			CTTAGACCGGCTCGGAG		TTCCACACACGACGAAAGAGCGAGAC	P	TTCCACACCGGAAAGAGCGAGACGACGACGCGCGACGCGGCGCTCCGA
496	1083	CTATCACTCAGTCAGATATATATGTTGTA		P	TCTAGACAAGTCTAAATATAGAAAGAAAGTCAAA		CTTGGTATACGTGCTCTTAAAAATGG	P	TGGTATATGCACTCTCTTAAAAATGGGGGTATTTTATATGATGATTAATTAAC
497	1085	GAGAGGCGCTAAACAGACGCTTAATTT			TTCTTTAAGCCTCGACAGTTC		AGAAATTAATTTGATTTTATTTTAA		AGATTTCTTCAATTTCTTCAACAGATTAATAAAAAAATCAATTAATATTT
498	1086	GCAGATGCAATATCTTTTCATTTT			AGCACAAGAAATTAACACTATGAGAAAAA		AGTTCTCTTCAATTTTCTTCAATG	P	AGTTCTCTTCAATTTCTTCAACAGATTAATAAAAAAATCAATTAATATTT
499	1087	ATTGTATTTTCCAGTCCATCTAGACTGCT			GGAGAGCTAGCTGAGGGTGG		TCTCTTCTGGTGACGCTGTGTGCTTCG	P	TCTTCTGGTGACCACTGTGCTTCCCTCTCTAGGGTTTTCCAGSCCCCCA
500	1088	TCGAGTCCGACGACTGCTGG			CTTGAGTACAGATGACATGATATATGG		GTGAGCTGGGAGATGATGTAAGCTTAAGCCCT	P	GTGAGCTGGGAGATGATGTAAGCTTAAGCCCTCTAGTTCGAGTCAGCCAT
501	1089	GGATCTCCGAGTTTCAATTATGTT			AAAATTCAGCTTTAGGCGGTCA		ATGAGAAAAGTGTGTAGACTCTACAT	P	GAGAAAAGTGTGTAGACTCTACAGCTCTACAGCAATAATAATGCAACGATG
502	1090	TTCCCTCAACCCACCCC		P	CATAAAGCAGATTTGGTGTGTG		GCTCACTGGAAAGATXTCTGGAACT		TGCTCAACATGTTCAACAAATCTCATGTTTCCAAAGGATCTTTCCGAGTGA
503	1091	CAGCAATTTGGTGTAGAGCA			TAAAGTTTGGGCAAAATACCCA		CAAAAGTAGCTGTGGCTGCAACACA	P	CAAAAGTAGCTGTGGCTGCAACACAGGAGTTCATGTTGATTTGTGACTGA
504	1092	CCCTGTTTCTGGGCTCCACA			TTTTTCTCCTATTGTATGTAGAAACTG		TGTATCAACCTCAACAGCTCAATTT	P	TGTATCAACCTCAACAGCTCAATTTGCCCCCATATGGAACCTTGTACTAT
505	1093	CGGTACCGAGAGGATCTG			TTCTTCTGCTTTTGTAGTGTAGGAACATA		CAATCTCTGCTGTTTCTAAGTAGGCG	P	AACTCACTGCTGTTTCTAAGTGTAGGCTTATVACTTAGTTTATGTAGTTTCTC
506	1094	TTATATATAGAAAGCCCTGGT		P	CTTCTACAGCTTTAGGGCT		CTGTCTCTGGGATTTCTCTCATTC	P	CTGTCTCTGGGATTTCTCTCATTTGTTACTCTCTCTGTTTCAATTCAGAGGA
507	1095	TAGTTCGACGAGGCTAGNA		P	CTCTTTAAGTGTCTCTCCCTGG		TXCTGCTCGAAATAGAAAXCTCTAGT		ACGAGAAACGAGTTTCAACAGCTTAAGACTAGAGCTCTCTAAATTCAGAGGA
508	1096	CAACGACGCTTCAACAGATTT		P	CAGCGATTTGCTTCTATGA		CTCTGCCACTCTCGAGATAGTCA	P	AAGAGCCTGACGTTTCAACAGCTGTCACTATTTCTGCTGATCATGCTGAG
509	1097	CTTTCATTTTACAGCTTTTTTTTTC			CAGGGCAATGCTGCTGACC		CXAGCTGGGTGAGTGGTGGCATCT		AGCTGGGTGAGTGGTGGCATCTCGGCTCACTGCAACCTCTACTCTCCG
510	1098	TACCGGATCTTATATGATGCA		P	CATGACAGCTCGCAGGAAA		CACTAGCACTCTGAGTGCACCTC	P	CACTAGCACTCTGAGTGCACCTCTAGTCTCTGAGCTCAGACCAA
511	1099	CTGAGTCAACACCAAGGCA		P	CTTGAATCTCTGACTGCACCTC		TAATATATTCGCTGAACCTGATACAT		TTTCTCTGAGGTTCAATGGGAGAGTGTCTCTCCCAACATCTGATGTT
512	1100	CTTGAGTTCGAATTTTCTCTCTC		P	TTTTATCTGGGTTTCTTAATATTGC		GTATTCCTGAGTGTGAGGAGCAATC		GTATTCCTGAGTGTGAGGAGCAATCCTCCCTCTGAGTGTGATCTGCAATTAAT
513	1101	ATTAGAAATGCTCTCCGAGC		P	GTTTTGTCACTTGTCTTAACATGATG		TTCTGCTTCTCTTCTAXCAGCTCTGATC		AGAGATAGCCTTAAACATATCTAAAGAGATCAGAGTGAATAGAGGAAGAGG
514	1102	GGCATCCGAGACATCTC		P	TTTTATTCTAGAAATCTCTCTTTC		CAGTGTTTAAGGTAGTGTGGTGG		TTTGTAGCCTSTGACACTAAGATGCCCAACCACTACCTCTTAAACAGTGT
515	1103	GGGGATCTTTTCTGTTATTA		P	GTTTTAGACTGTGGGAGAC		TCCTGXAATATAGXCACAGCACC	P	TCCTGXAATATAGXCACAGCACCCTGCGATATAGCCACCAACATCAATTAAMA
516	1104	TGCTCAGGCCACCAAG		P	TCAGTTCACTTGCCGACTTT		AGGACACTGAGCTCTCCAAAGAC	P	AGGACACTGAGCTCTCCAAAGAGCTTTAAGCCGAAAGGATTCAGAGTG
517	1105	AACTGGAAGACGACGACG		P	CTTCCACACAGGTGTACCCAC		TTTTAAITCAITGAGTTATTTTGG		CAAAACAAANAATTAACAGAAAAACCAANAATCACTCAATGAATTAAMA
518	1106	CTGACATTTCTGAGGNTAATAACAA		P	AGGGCAATGGAGATTGCA		AGTCTGGGATTAAGGGAATAGGCGAC		ANTATCAGAGCTCTGGCGAGGCGGCTGCTCATCTGCTTAATCCGACGA
519	1107	AATCAGAGCTCAAGAGACTGTCTC		P	TTACTTCTGCTCTCCYAAAGTGT		TCCAATAGGGGCTTAAGTGAATTAAGT	P	TCATAGGGGCTTAAGTGAATTAAGTGTTCGACCTCAACATCTCTAGTTTGG
520	1108	CTAGAGTGTTCGAAAGCTAATAAGT			CTTCTGCTGATTTGTAGCTCAAAA		AGCAAGAAAAGAGCGCATTAAC		TAATACATTTTTAAAAAATAGAAATCGTTTTAAATTCGCTCTTTCTCTTCT
521	1109	GTTGTGTGTTTCTAGAGATTGG		P	ACAGAAATTAATCTAACAGAAAGNA		ATAATTTGGTTCTATTTCTTAACAT		AGCATCAACAAAAAATAACAGGCTTTAGAAAATAAGAACCAAAATAT
522	1110	CTGACATAAAGAGTCCACACATTT		P	TTGTGTGCTGGTTTCTCAATAATTT		GTATGGGAATTTGAGGTAAGG		AAACCATACAGNTTTTAAAAATGCCCTTTTACTCTCAACATTTCCCATAC
523	1111	CTTTTCAACACGAGGCG		P	AAGAGATAATAGGAATGTGTATGGAA		GTAAAGTTGGATGGGACAGACGCCAA	P	AAGAATTTGGATGGGACAGACGCCAACAATATCACTGCAATCTGAGCTTACT
524	1112	ACAGTTTGGAAITATGGGAGC			CATGTGCTCTGCAAGGGTCT		AAAAAATAAAGTAAAGACATACC		AAGAATTTGGATGGGACAGACGCCAACAATATCACTGCAATCTGAGCTTACT
525	1113	ATTCCCGGCCATGCTTAAC		P	TACATTCGAGCTGGGCA		AGATATCTGCTCATTTTCTXTTATAGGT		CTTTCTCTTATAAATTAACCCAGTCTCGGGTATGCTTCTTACTTTTATTTTTT
526	1114	GTGGGCTCAATACTACTGCT		P	TGCACTAGTATAAACACATTCGAGATTATCC		TCAGAGCTTTTATGATCATTTACACTATACACTATAGGATAAATCAGATTAAT		TTCCAGAGCTTTTATGATCATTTACACTATACACTATAGGATAAATCAGATTAAT
527	1115	GAGCTATCTATGACAAACCCACGA		P	TGGTGAGGTGGGCATCC		CATCATACCAATAAAATCTCGAAG	P	CATCATACCAATAAAATCTCGGAAG
528	1116	GAAGAGCGCATTTCAITTCGAGAC		P	TGCGAGATAAAGCAGAGAGG		AGTGGAGGAAAAAAATCAAGTTA		AGTGGAGGAAAAAAATTAAGTAACTCGAGTTTTTTTCTCTGCCGCT
529	1117	CATTTGTAGTCTCTTTACAGGCC		P	TGGCGAAGCAGCACAGTG		GCXACCATACCATGATGTTTACTTTT		TGTTCTCTTCAKAGTTCAGCAAAATACAGAAAGTAAACATCATGATTAAGTTT
530	1118	GTGACACATCAGTACATTTTAAAGTCT			ATGCAACCATACCATGATGCTTTTAC		TGTTAGCTTTMTTATTTTCTGACT		TTACAGGCCCATGTGTTTCCCTTTCCAGGCTTCGAGGATGAGAAATAAANAAGTAANCA
531	1119	GTCTCTCTTTTCAAAATCTAGAGGAT			TGCGAAGCACTCTTTTTTGGC		AAAAGTTCGAGCTCAAAACATCTCAT	P	AAAAGTTCGAGCTCAAAACATCTCATCTTTTAAAAAATGTCAATTA
532	1121	CATCTTTTCTAGTGTGGCTTATCTTT		P	TGGGAAAGTGAATGAGGATTT		TCAACAATTAAGCTGATATTTAAGA	P	ACACAATTTAGCTGATATTTAAGAA
533	1123	AGGTGAATGGGAGCAGACATAT			GTACCGGCAATCTGCTTGT		GGTGCATCAGCAGAGAGAGCTGCA	P	GGTGCATCAGCAGAGAGAGCTGCGAGGAGGTGCTACACACTCTTTTAA
534	1125	GCACAAATGTCGCACTC		P	CTGCTGTATTAACAAATATCTGACACTGG		AAAAACAGAAATGTACTTCTCAGAGT		TTGATCTTGACTTTCTAGCCTCTCCAGAACTGTGTGAGAGTAGCATTTCTGTCTCT
535	1126	AAITCAAGATAGCAATTTGGTGG			GAAITRANAATGACAGACGCGGC		CATGAAGCTTAGGTGTACAGATTTTTG	P	CATGAAGCTTAGGTGTACAGATTTTTGAACAATGAGACAAATCCACCCACAGAC
536	1127	CACAGGCAAGTAAATCATTTTGTG		P	ACAGTTTTCAAGGTTAATAATGACTGCTT		ACTGTCTCCAAAAAAXCTGATGACA		TAITTTCTGAATGGGCGAAGAAGTATGTCTATCGACTTTTTTTTGGAGACA
537	1128	TCCTAGATATACCCTGGGTT		P	CGGGCACTCTGCTGGGTT		GGCTGGTGAGCCTGGGCACTTGGTGGCAACTCTCTCTGAGAAAGGAGGAG		GGCTGGTGAGCCTGGGCACTTGGTGGCAACTCTCTCTGAGAAAGGAGGAG
538	1129	CCGCTATGGAAATTTGAGTACTTT		P	ACAAGTGAATGCTTACTTAAAAACTGAATTA		CTAAAAAGCTGAATAGCACTTCGCG	P	CTAAAAAGCTGAATAGCACTTCGCG
539	1130	TCGTGCTGTATGTTGCTGTGTGG		P	CNAGACAGCTCCGCCCT		AGTATTTGAGAAAGCCAGACTTTTTT		AGTATTTGAGAAAGCCAGACTTTTTTAAAAAANAATTCGAAATTTCTCAGGGCT

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	Modified	3	GBA primer	4	Planking sequence
540	1131	ATTAAAGATCGAGTGGGAAGAGCT			TTATTGTCTATGCTTCTCTCCATAAATAAAG			P	TTTAGAGTGCXGCTGTGTGTCXGAGGCA		TAGAGTGCCTGCTGTGTGTCXGAGGAGTTTCTTAGATGCGTAGGTATATAGCTG
541	1132	AACATCTTGTGCTGACTGCTACCC			ATGCATCTTTCTTAATGCTTTTCCATGAA			P	AAAGACTTTAGCAGCAGCCACAATAA		AAAGACTTTAGCAGCAGCCACAATAACAGTGTAGAGTGGAAACTTTTAAACACTTTCAATCG
542	1133	CTAGAGAGTCTTTCATATTTTCTCTTTT			TGCATTTCTCCCGGAA			P	CAATTTCTACAAAATAAACCATCTTGG		CAATTTCTACAAAATAAACCATCTTGGATTTTAAATTTGATTTGCAATTAATTAAT
543	1134	GTTCATCTTAGCATAGTATAGTAGTAAT			TGGAGTGTGCTCTATCATAGCC			P	TTTAAATAATTTGCTCTTCTACCTTTT		TTTAAATAATTTGCTCTTCTACCTCTGCTCTCTATGATG
544	1135	CCACTCTCAGACTTTTGTGTAATTTT			ACTTGCTAGTGTGGGATCACTTTTAA			P	AAATACAGCAATAGATACCAATAT		TCACTCCACTGATGTGACCAAAACAGATTTTGGTATCTATTCGCTGTATTT
545	1136	ATTTTTGCACGTGTTATTTCTCTCG			CATYGGGTAAATAGACAGCATTTCAA			P	ACGAATTTGCGATTTGCAATGAGAGCTAC		CTGCATTTGCGATTTGCAATGAGGCTTGTGTTTAAAGGCAATTT
546	1137	GATTCCTGAGGCCAGAG			ATAATAAACCCGAGTATACCAATATTTA			P	CATATTTAGTCTTTCATTTAAACAA		TAGAAAACCACTTCTCATCATTTGCTTTTAAATGCAAAAGGCACTAATAA
547	1138	ATCTCCACACACTCTCTCGATC			GAAAGATGTGACAGCAATAATCAATATAGG				GGATGCAATGTACAGATTTGATGAGCTGTG		GGATGCAATGTGATGAGCTGTGTTGCTGCTGCCCTACAGAA
548	1139	GATTTAGTGATGATATAGTTATTTAAGATG			TCATTAGTCTTAAGTTTTCGAAATGTG				TAATTCAGTACAGACAGACTCCCTTAAC		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
549	1140	CGGGATCTGAAAGGCCA			TAAATGTGTGCTGTAACACAGG				ACAGCATTAATTCGCTCCGCTTGGCTG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
550	1141	TAACTGTTAGCAATCTTCAGAAATTC			CTCTACTACAGGAGGCTCAGAG			P	ACAGCATTAATTCGCTCCGCTTGGCTG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
551	1142	GACTCTGAGCTTGAAAGCACTCC			CTTCAATTAACAAACAGCAGAGCC			P	TCATTAATXCAAGTCTCTMACTGTAGCA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
552	1143	CCGTAATTCGATCTTAAAGCTATAGAA			TGCGACATAATGCTCGG			P	CCTCATGATCTGCTAATCTCCACTTC		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
553	1144	CATTTCTGTCTGTAATCTGCGCTTA			TAAAGGTACCCAAATGAGAGTCA			P	TACCGAGTTATTTTATTTAGTGTGATG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
554	1145	GCCAACTATCTTAAAGCTATAGAA			CGAGGAAATCGAAATTAAGATG			P	CAGTGAGATACCACTTATTTTATTC		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
555	1146	AGCAGCGGAACCTCTTACGTTT			CATTTAAATAGCTACTTTCCAGGGG			P	AGAGTCCCATCTGACTCTGTGATTTG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
556	1147	ATCTAGAGAGTCTGTAAATGACTATCC			ACGTTGAGCTCAGTATGCGAGG			P	AGGCTGGCATGCTGATACCTGCTG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
557	1148	AAATATGATAGCTCTCTCTCATATAT			GATGATTCGAGGTGGGCA			P	CXGKTCTGATCTACAGTCTGCCCTTA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
558	1149	CAGAAATATACATTTCTCTATCTGCA			ATAGTTTCCAGTCTTCTGCTCTTGTGTC			P	TCAGTACTTTCTCAGTCTCAGCA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
559	1150	AAATGCGCAGAGGATG			CTTCTCTGATCTTGAGCTCT			P	AGGCGTTKCAACAGGGCATTTGCTT		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
560	1151	GAATGAGTCAAGATAGCAAGTCTATAATACA			TGGAATTTGTCGAAATGCTAACTTG			P	TAGCAATTTCAACAGAGGCAAAAA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
561	1152	TACGGGATCTGCTTAGC			CAGCTCCACATGACGATG			P	CTACACTGCCTCTXTAAGAACT		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
562	1153	ATCCGATATATCTCTCAATGCAC			GACACCAAGTATCTAAACACCA			P	CTGTGCTGCTCTCAGCTCTTAGACT		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
563	1154	ATAAGAGGTGAGCTGTAGGACCA			CMAATACAGGAGATTTTCTAATGATTTT			P	CXAAACAGGAGAGAGAGGAGTCC		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
564	1155	ATATCTGTAATCTGTAAGCATAGTAACACA			GGGATCTCTGCTGATCTCCCT			P	CTATGAAAGTTAAACAGAGTAGAA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
565	1156	TTACTGTAATCTGTAAGCATAGTAACACA			TAAATTTGCGCTGATCTCCCT			P	AACTATGTTAAAGGCTXAAAGTTTCA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
566	1157	TGGTTATAGTCAATAGTAAAGC			GTATTTAGCAATTTCAAAAGTAGACCT			P	CTCCTACTGCTGCTGTTXAGTCTTCT		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
567	1158	TACATCTGATCTAGTCAATATTTGA			CGCTATAGATCTCTACAGCAATTC			P	ATATTTATXCATTTTTCGATTTTAT		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
568	1159	CACAAAGCATAGTCACTTTAGTAGCG			AGTGGTTATAGTATTTTAAATAGCTTG			P	TAAATAGCTTCTGCTGCTCTCTCTA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
569	1160	CCTACAAAGCAATCCCTG			ACGCCCTGCTTAAGCT			P	TCCTCACTCTAATAAGACATAC		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
570	1161	CAGATTTAAAGGAATGTTGABAATAC			CTGCTGCGCTGTGAGAACT			P	CAATATCATTTGTAAGTTTCTGAG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
571	1162	CGATCTTGGCAGCGCTG			CCAGTAATAGGAATTTCTTGCGTT			P	GXAAGCTGTGGAARTTTCAAAAGAGTT		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
572	1163	CTGATTTCACTTATGCAATTTAAGAA			TCGACGCTGCATCCGAC			P	TTTTCCGACCAAAAGCAACTAATA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
573	1164	CTGATCTTCTGTTTAAATGG			CTCTGCTGCGCTCTCTCG			P	TGTCGTTGTCCTCATAGATGGAGCCCTG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
574	1165	TCNCTGTCCAGAGATAGTATGGG			CTCGNATGTATCCGATGT			P	GAACTGCCCCCATGAGCCCATAC		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
575	1166	CAATCAGTCTCCACAGAGT			CTGATATAGTGTGGCTCTGTGT			P	TCGCTCTGTGGAXCXKTGAGATACCA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
576	1167	TAATATCAAGAGAGGCTGCTGTCTTC			CGTCCCACAAAATCAGCACT			P	ATTCACTCTGMAAACTTACCTTTCT		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
577	1168	CTGGGAAGGTGAGGTGA			AGAGCGCATAGTTTTCAGGTAGTCTT			P	CTTCAATTTTCTTCAATTTTCTGTA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
578	1170	GCTATCCAGCAGATATCATCATATTA			AGAAATCGCTTTTTCCTCAGCG			P	ATTTGATAGTGTGTTATATXTAGCCGAG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
579	1172	ATAGCAAGTCACTTTACTTCGATTTG			GAAGATGTGGGAAGTTTCCAA			P	AGACACCTCTGCTCTCACTTCACT		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
580	1173	ACCACTAAGCTCAGCTATCATATTTTG			CACCTGGGAGCGCAAGC			P	TATTTTATGATAGATAGTGGCTTTCA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
581	1174	ACACAGACCCCCCAGAGG			CTTGTCCAGACTCCCGGG			P	ACTTAACGAGAACTXGAGAGGCCAA		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
582	1175	TATGCTACAGAGAGTCAAGCA			CNAGGCAATAATTAGAATGCCAA				AACTCTTCAATTAATAGAGAGAGC		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
583	1176	CGATATGACATTTCTCCAGAAATAG			ATTCAAGTACTGATATGTTTCCAGTTTT			P	TTCGAGTTTGTGTTCTTTTGGCTCAG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
584	1177	CGGTACANNNGGGATACATTTT			CGTCCGATAGCTGATGATGAGTTT			P	CAAGAGAGCTGAAGAAAGAAAGTAG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
585	1178	AGGCCATTTCCCGACCTG			CTAGAGGCTCAGAGCTTTTCTG			P	TGTACAAATGCGCTGCCCGCCACAGC		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
586	1179	CGAGCCAGAGACTTATTTCTCATCTAG			TGGCTACATCTTTCTCCATATAG			P	CTAGATAACATCTTTCTCCATATAG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
587	1180	AAACAAACATCCAGAGCGCTG			GTCTGCTCCTCTGCTGCTG			P	TGGGGTCTGCGCTCTCTGCTGCTG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG
588	1181	AACTGTCAATAATTTGAGAGCGCTG			TGCTAGCTGTGTAACAGAACCAATAC			P	ACTCTGTGTAATAATTAATTAATTTCTG		TTTCTAGGTAACAGAAAGAAATTTGCTGTAAGTAGAGCAATCCCAATATGCG

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	3	Modified	Flanking sequence
589	1182	TTGAATTGTAGCTCCCATTAATTACAC	P		CTACCAAAAGAAATAGCATGGGCA	P	GGAAAACTGCCCCACATTTTCAATTTC	GGTTGTGGCAGGGACCCCGTGGCAGGGAAATGGAATGTGGGGGCGAGTTTCTC
590	1183	GGTTTAATACGTGATTCATTTATTAGCTGTAG	P		AGAGATGATAGTATCTTCGAGGTAGAGAAAC	P	TAGAAAACTGTAAACCCCTAAACCCCT	AGTTTGTGVTGAAGGGGGATCTGCAGGGTTTAGGGGTTTAGAGTATTTCTTA
591	1184	TGACGCAAACTGCTTGGAATG	P		TCCGAATGGGAGGGGGCT	P	TATTCGCTGTCAAGTTTGTGTTTCAAC	TATTCGCTGTCAAGTTTGTGTTTCAACGGCTTTCATGTTTCAAGTTCCTTTGC
592	1185	TTTTTCTCTCTCTTTTATAGGTATGG	P		GATCAATTTTCAGGCCGACATGC	P	TTTGGCGCAGCTCGCGTCCAGTTCAGTAGC	TGGCGCGAGGTCGCGTCCAGTTCAGTTCAGTTCGCGTCCAGTTCAGTTCGCG
593	1186	AACAGCTCTTCCATATGTTTAACTCC	P		CATTTTGTCTCTCACTGCAATC	P	TCCTXTCTTCCAGAGAGCGAGTCAT	ACCTGTCAATGTATGAAAGTACCAAGAAATGAGTCTGGCTTCTCCAGAGGAG
594	1187	GTGTTGTGTGCTGCTGTTTATATGC	P		AGCCATGCAATTTCTAGCA	P	GAAAGAAATGTAGGCAATCATAC	GATGTTTAATAGTCTTGTAGTATATCCGGTAGTATCATGCTCCATCATTTAG
595	1188	GCTGGGGATAGTATATGCGA	P		ATTCAAACTCTTGCCCATTTTTC	P	TTCTATTCAGCTGCTCTTTTCTTAC	AGTCTAGAAAGATATTTATAGATAGGTAGAGAAAGAGGAGCTCCATATGAA
596	1189	ATCTCAGCTCACTGCAACCTC	P		CATATGAACCTCTGCTACTAAACATC	P	GCCTCCCAAGTTCAGATCAATCTCC	GCCTCCCAAGTTCAGATCAATCTCCCGCTCAGGCTCCCAAGCTAGCTGGGA
597	1190	GAGCTGGAAGCAATCTCTGCTAG	P		ACCGAGGGATCAATCTTTT	P	AATATTTAGAGATATTTGATAACTTTC	GTAATGCATGTCAGCATACGAAGTTCGAAGTATCAAAATATCTTTTAAATA
598	1191	AACATAGGAGACCCCTCC	P		CAGAGATCTGCTGCTGCTCAG	P	TAGAGCTCGAGSANTXCTGCCATCAAC	CCAAAATAAATAAAAAATATAGTCTAGGCTGTGATATGTCACATGCTCGAGTCC
599	1192	TGAGCGATCTGGATTTTCTCG	P		GAATAGCTCAGTCTGGGATTTTCTTC	P	CAGTGTAGAAATATGATATACAAG	CTCTGCTCTGAGCAGCATAGTAGTCTGTGATATGATCAATCTCTCAACTC
600	1193	CTGGGACCAATATACAGCTACT	P		CTGTGCTCTTGAGAGGGTT	P	TCGAAGCTXTGTGTGCAGAACCCAGG	CAGCTCTGTGTGCAAGAACCCAGAGGCGCTTCTGGAGTGCAGTCCACAG
601	1194	CACCAATTACCAATTTCTGCA	P		ACCATGATTTTATTTTCCAGTA	P	TTATTAACCTGTTGTGTTTCTCTCT	TTATTAACCTGTTGTGTTTCTCTCTCTGTATATGATCAATCTCTCAATTTCTCAG
602	1195	CAGACAAATATATACCTGTTTCCA	P		TCACGAGATTTTCACTAGAA	P	CTGTGCTCTCTGAGTGTGTTCTACCTC	CTGTGCTCTCTGAGTGTGTTCTACCTCTGTATAGGATTTCTTAAATTTCT
603	1197	CACCTTCACTTATTAAGACATATAGACTTAAG	P		ACCTAATATAGTATACAACATTTCTGCTCACTCT	P	CAACTTCTGCTCACTCTGATTTTCCATT	CAGCGATTTAAAGACTCTCCACAAATAGCAATATGCTATCTGCGCAATTTGAGCTT
604	1198	TGTGAGTGTGAGCATGCTC	P		GTGAGGCTGMAAGTAGACGA	P	AAGTACATTTGTATAGAGAGTAGAA	GTTAACATTTGTATAGAGAGTAGAACGGAATATTTGGTCTGCTCTCTCTCTG
605	1199	GGCATAGACAGACATACGAC	P		TTCTGTGCACTTTGTCCAAA	P	TTGAATTTTGGTAGGATTTGATTT	CCAGAGAAAGCCCAGAAAATAAATATAGTCAATTTATGTTGTGGCAATTTGATTTT
606	1200	AATATCGTGTTCATGATATGGA	P		ACTGTTAGGGCTTTTGTAGTTCC	P	TTGAATTTTGGTAGGATTTGATTT	TACTACTTCAAGTATCGACAGGTTGMAATACATCTCCGTAAACCAATTTGAA
607	1201	TCCAAACATTCGTTCTATGCT	P		GAACATTCCTTTAAGCAAAAGC	P	AAGCCTATTCAGAGCGAGACGCTTA	TACCTCTCTCAGCGCTTCAGAGAGGTAGGGTCTTGCTCTGATATAGGCTTT
608	1202	CCTTACACTTTTATGTTATGAACAACACTTG	P		TGAAGCTGAGAGAGGTGAGTAG	P	CCTTAAATCTCATGAACCTACCTCT	CCTTAAATCTCATGAACCTACCTCTGCTAGTTTGCNAACATTCCTCTATGCG
609	1203	ATTAGGTCACTGCATCATGGCA	P		GAATCTTTCTGAGCTCTTAATGCC	P	CCGAGCTCTACTCCGAGAXXTAAMAGGA	AGTGTCCCAATTTGGCCTATGCCATAGAGTCTTTTAAATCTCGGAGTAGAGGCTTA
610	1204	CCATGTAGTAATATATGCCATGTC	P		AGTCTCAGATATCTTCACTAGAGAA	P	TAAACAGTGTGAGCAATGGAGTAATA	TAGTTCAGATGCGCCCTTGAAGATATGATATAGTATCTTCACTCTCCACACTGTTA
611	1205	CCAACTTACGCAAGATAGGA	P		TCGACACCTCTTCACTCTAGAAA	P	TCGCAAGAGAGAAACAAATCTATT	GGTAAAGAGAGAAACAAACTATTTATAACTATTTATCAATATCATATAACATTTTCT
612	1206	TCTGTCTATGGACAGCCATTT	P		CTGACAGAGGAGGAGGATG	P	TCCTTCACTCTTGGCTGTGCTGTCA	TCCTTCACTCTTGGCTGTGCTCTTTCAGTATCTTCTGAGTGGAGCCACAGAC
613	1207	AAGCTAGCCCAATGCCCT	P		CTCCTCTCTCTACATATGCCCTTAA	P	TCCTGAGTGGGCAATGAGGTAGAG	TCAGATGGTGGAGCTATCTGCTCTACCTCTCACTCTCATCTCCCATCTAGCA
614	1208	GTATACATATATACATCTGTATCACCCAA	P		TGTGCTCGGGGTTGTTG	P	TGCTGGGTTTGTGTTTGTGTTGTCAGG	GCTTAAVGGAGGGTACGTTTTCMACTCTGACATGCAAAAACAAACACCCCAAC
615	1209	GGCTAATGTTCAAGATTTATTTACTAGC	P		GGTTTGGGTTGTTGTTT	P	ATAACATCTCTATCAACCAAGGTAA	AACATCTCTATCACCCAAAGGTAAACGAGGGAGTCACTTCTCCMCTGTA
616	1210	TCCCTCCACTCTCCCAACC	P		CTAACCCCTCTTGTGCATCTTATTAATAC	P	TCATCAGAAAGATAGCACTCCAC	TCATCAGAAAGATAGCACTCCCAACACAGATCTGAGGTGGAGGTAAGATGA
617	1211	TCACCTGTCATGGGCAAC	P		TGCATCTCATGTCTTGTCTATG	P	GGCTGACTCCATGTCTTGTCTATG	GGCTGACTCCATGTCTTGTCTATGGTCCATGTCTTGTGATATGGCAATAG
618	1212	ATTGGTAGGAGGCTGAG	P		GATCTGAGATGCTGTTCTTTTGC	P	CCTTGAATATCCGGAATCTATGG	CCTTGAATATCCGGAATCTATGGCGTGGATGACGTGGAGAGACTGT
619	1213	GGTAAAGGTAGATGAGAGKAGTCTC	P		AGNCGCTTGGCTTTTGT	P	AGAACAACAGAAATTCATTAATAA	AGAACAACAGAAATTCATAAATAACCTACTATCAATCACTGAGTTAAACAACA
620	1214	CGAATTAATATATCTCATTTGATAGAGTATTG	P		CAGTCCCTCCCGAGCGATG	P	MTTCTTCTTAAAGTGTCTCCAGTCT	GTCAACTCTCTCATTAAGCAACCCGCACTACTACCACTCAACAAAGAAAT
621	1215	ATGGCTAGGAGATCAGACTCTTT	P		GTCTGTATCTTTTTCAGAGCTTAGAT	P	TAGATAGGAGXGCTTGTCTCCAGTCTG	GCACACTTTCGCTGCTCAGCGCGCCACAGGCTGACGAGCTTACAAAGAAAT
622	1216	CAGCACTGCTTTATGTCTCC	P		ACCGGGAGTCTTTTATGCTATG	P	GAATTAATTAAGAATAGAAAT	TTAACTATTAGATAAATATATTTCTGTACACCATTTCTATCTTTTAATTAGA
623	1217	ACACATCATCAGCATCAGCAAGA	P		CCCAACAATCTGTGCGATGAATAAG	P	AAATTAGAGAGAGAACACATAGTGT	CTCCTTTGAGATCTCTTTTGTCTCCCACTATGTATGTTCTCTCTCTTAAAT
624	1218	ACTGTTGGGAGGCAATATGG	P		CTCCCAACAATATGATATATCAGG	P	GTTTGAAXATGTGAGGACATGAGATTT	TTTGAAXATGTGAGGACATGAGATTTGGGAGGGGGCGAGGGGCTGATCATAT
625	1219	AGCAAGCACTAATACAGTCAACTGTACC	P		CTCTGCTGCTTTTCCAGTT	P	AGTTTCCXAAGTCACTTCCXATTTTGTG	CAGTGGCGCTTCTGAAAAGATCTGGAAAAGACATATGGAAGTCACTTTGGGA
626	1220	GACTTATTCACAAATTAACATGMAATGC	P		TGGTTCTCTCAAAATGAGACCAAT	P	GCTTATCTGTCTGGTGTAGAGTGT	TTTTTTAAACATCTCATCOMATTTAACAACACTTCTTACCCGACAGCATAGGC
627	1221	GTTAAAGTGCATCTTTCAGGCTCAC	P		GCTTCAGAGCAAGAGGTT	P	GAAAGCAAXXACAGAGCTAGAAATCTTA	TACCTAAATTTGTTTGCNAAGGTAGACATAGAAATCTTACTTCTGATTTCTCCCT
628	1222	CAGGGATACATGCGGA	P		CTATTATACAGATACCAAACTGGCTTAA	P	CTCAGAGTCTGAGGCTGGGATGT	TTTGGTCCCTGATCCTCOMATCTTAGACATCCGAGCTCTCCAGAACTGTGAG
629	1223	TCAGCGCATCTCACACCTC	P		CATTTGAAGCTCAGGATTTGAGAC	P	TAATTTTGTGTAATTTTTTATAGAAAC	TAATTTTGTGTAATTTTTTATAGAAACAGAGTTCGCGCACTTGCACAGTCTG
630	1224	GGCTCAGTCAGGCTCTCT	P		CCACTCAAGCCCTGTGC	P	TGCAAXGCTTCTGGATACACCTCAC	CNAGGCTTCTGGATACACCTCACCGCTACTGTATATGCACTRCCAGTCCCAT
631	1225	AAATAGGCAATTCAGAGCATGAA	P		GNAAATGGAGTATGATTTAGAAATGC	P	GTGTTGGGATTTGATGATTCGATCT	CCATTAACCCAGAAATCTAAATATATGATGCTCTCTTCTCTCTTAATCCCAAC
632	1226	TATCATCTCAGCAGACTGTGAT	P		CAGGCTTTCACCTTCCCTC	P	CCTGTTGGGATTTGTGCAATGAGAG	ATGATGGGTTTCTGGCTCAGGTTTGCATCTTCMAATCTGCAAAACTAGCCAGG
633	1227	AAAACTCCACATGATTTTTCAG	P		GTTTTCTTCTCTGGAAATGTTGA	P	CCTTGGAAAGAAAGTAGACACTGGT	CCTGGTAGTTTACAGACTCCAACTGGGACCGATGCTCTTACTTCTTTTTCGGA
634	1228	TATCTTCAGAGGAGTTCGGTGG	P		CGGCGAGCTCTGTGAAAC	P	GTCTGTGAGAGCCCAATGAACCCCTTT	GTCTGTGAGAGCCCAATGAACCCCTTTTGGAAAGTTTCTCAGATGGCCCAATGAGA
635	1229	ATCCTCCCAACACTTTTTC	P		AAATTTCTCTTGTATATCCAAAGTG	P	ATCAGTAGGCTCTTGTGTGAGAGAAAGTAGATGGAATGGCACTTCCAGATATAGCA	ATCAGTAGGCTCTTGTGTGAGAGAAAGTAGATGGAATGGCACTTCCAGATATAGCA
636	1230	GCTTTTCCCAAGGCTTGGAAATG	P		TGGTATTCGAATGAATGATTTTTCAG	P	ATTTGTATACTCATCTACTAGTAGTATGATGCTGCTGTTGAGCTGGAATAC	TTGTATACTCATCTACTACTAGTAGTATGATGCTGCTGTTGAGCTGGAATAC
637	1231	AGGAAGCTCAACCAACAGAT	P		TTTTCTTTTTTTTAAAGACGGGTCTC	P	CTTCAACCAAGAGGAGGAGGTTGTAG	TCAACCAAGAGGAGGAGGTTGTAGGAGGCTGTAGATCAAGCACTGCACTCTC

[illegible]

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	2	Modified	3	Modified csa primer	3	Flanking sequence	4
687	1284	CACAAATCAGGCTCGATTTTACAAA	P	TGCATTACACTAGTATCAGAGAAATACAGAA	P	AGCATGCTGCTGTTTGGTGAATTCCTCATG	P	AGCATGCTGCTGTTTGGTGAATTCCTCATG	P	AAGTCTCAGCCXTTTAGGACGTAGATAT	AACCTTGAANAATACTTCTAACTTAGGCAATGGAAATTTCAAAACAGACAATTCCT	
688	1285	TATGTGACTTGGCTGTATCTCAGAC	P	CATTTCTCTGTAAATTTTCACTCTCCTC	P	AATGCTCAGCCXTTTAGGACGTAGATAT	P	AATGCTCAGCCXTTTAGGACGTAGATAT	P	TAGTCTCTTAAANAATTTTCACTCTCCTC	CNAACCTCAGGTTCTTAACTTACTCATATCTAGTCTAGTCTCTAAAGGGCTGAGACA	
689	1286	TGTATATCGGTGACAGAGGAGTCTAGTCT	P	ATGCATCTGGAANAATAGGAGTACTCTT	P	GTGATCAAGCTGGGGTGG	P	GTGATCAAGCTGGGGTGG	P	AGTGTGAGCCGCTCTGCTCGAGAG	AAACCTCGGCCCTTCACTTCTTAAATACATCTTCTTCTCCAGACAGGGGCTCACACC	
691	1288	ATGAATGAATGAGCAGTAGGAG	P	CAGATAATCCCAAGACACTCAGACC	P	CAGATAATCCCAAGACACTCAGACC	P	CAGATAATCCCAAGACACTCAGACC	P	CGCTGTGACXAGGTGTTTGGGGACAG	CTGTGACAGAGTGGTTTGGGACAGACGCCCTCTCAGATAGAGCCCAAGGCTGTG	
692	1280	CGGCTCGTATTCCTCTCTAAAAA	P	GGGATCAGATTTAAAGACAGATAGATAGTATATGC	P	GGGATCAGATTTAAAGACAGATAGATAGTATATGC	P	GGGATCAGATTTAAAGACAGATAGATAGTATATGC	P	AGTATATCCAGCAGAGTCTATAGAGCC	TTTTCTATCGGCATCGCATATATTTAGATGGGCTCTTATACATCTCTCATATACT	
693	1291	AATGAGCCCACTATACGAAA	P	CATCAGACCAACCAAAACCTG	P	CATCAGACCAACCAAAACCTG	P	CATCAGACCAACCAAAACCTG	P	CTCTCXACGCTAGAGTCTCTGTGGG	AAAGTCTCAGAGGGCTTTCTCGTGGGGCCCAACAGACTCTCTCAAGGGGAG	
694	1292	AAATCTCATTTCAATATGACTCCCA	P	CATCGAGATCTGTGTCAGGATGTTCA	P	CATCGAGATCTGTGTCAGGATGTTCA	P	CATCGAGATCTGTGTCAGGATGTTCA	P	CTCATCTATGTGATCGGATTAAGCTGACAGAGCTTACGATCATCATGAG	AATCCCATATGTCAATCGGATTAAGCTGACAGAGCTTACGATCATCATGAG	
695	1293	AAATCTCAGGTTCCCTCCCA	P	CTAAATGCGGAGACAGCAGAG	P	CTAAATGCGGAGACAGCAGAG	P	CTAAATGCGGAGACAGCAGAG	P	AGAGCAGCAGGCTGGGGCCGACAGC	CTAGTCTCTGCACATCTCAGCTCCAGCGGTGTGCGCCGACGCTTGGCTCTCT	
696	1294	AGCTTATCCAGTACTCTCTGAAAATAA	P	GGGGCATATGTTTATAGAGGATTTCT	P	GGGGCATATGTTTATAGAGGATTTCT	P	GGGGCATATGTTTATAGAGGATTTCT	P	CACTAAACCTCTTATATCTATATGTGGCC	CTAAACCTCTTATATATGTGGCCGCACTTGGCCACACAGATATAGCGAG	
697	1295	ACGATTAATTAATCTGCATCATATGG	P	TAATAATATGTGATGTTTATCTGACTGATC	P	TAATAATATGTGATGTTTATCTGACTGATC	P	TAATAATATGTGATGTTTATCTGACTGATC	P	TGAGGAGAGGTTTTCAGGGTGTTA	TGAGGAGAGGTTTTCAGGGCTGTTTACGAGCAAGGTTGGAGACATGGCG	
698	1296	AATCAAAAAGAGGAGGAGGAGAGA	P	GAAGCTGATGATTCGAAAACCTCTC	P	GAAGCTGATGATTCGAAAACCTCTC	P	GAAGCTGATGATTCGAAAACCTCTC	P	AAACGTCGAACCCCACTCGACTTACT	CAAGCAAAACAGATAGGAGAACACAGATAGTCTAGGCTGGGTTTCAGCTGTT	
699	1297	ACTTTGTTTCTGCCAATCCA	P	CATAGAGGCTCCCTGAGCTCCAGAC	P	CATAGAGGCTCCCTGAGCTCCAGAC	P	CATAGAGGCTCCCTGAGCTCCAGAC	P	GCACAGATTAATGGGGAGAGTAGAC	GCACAGATTAATGGGGAGAGTAGAC	
700	1298	TATCATATATTAATATACACGAAAACAGACAG	P	AGGCTCTGTTTAAATAGCTCGA	P	AGGCTCTGTTTAAATAGCTCGA	P	AGGCTCTGTTTAAATAGCTCGA	P	CCCAAGGATTAAGGGGAGGAGGAGAC	CCCAAGGATTAAGGGGAGGAGGAGAC	
701	1299	GGGACAGCGGAGGAGACT	P	CTTGCAGGACAGCTGGCTT	P	CTTGCAGGACAGCTGGCTT	P	CTTGCAGGACAGCTGGCTT	P	CTCTCGAGTCATGTTCCCGCCGACG	CTCTCGAGTCATGTTCCCGCCGACG	
702	1300	TATCTACTCAGAAACACGCAAA	P	CATGACAGAGGTTGGTCTATGGC	P	CATGACAGAGGTTGGTCTATGGC	P	CATGACAGAGGTTGGTCTATGGC	P	TTCTAGATGGCTGTCAGGTTGGTGG	TTCTAGATGGCTGTCAGGTTGGTGG	
703	1301	TGTTCTCAGACATAAAGATCAGGAG	P	CATATCTCTACAGAAACCCCAATG	P	CATATCTCTACAGAAACCCCAATG	P	CATATCTCTACAGAAACCCCAATG	P	TTGGTGGCCAGGCACTACTACTGTGCT	TTGGTGGCCAGGCACTACTACTGTGCT	
704	1302	TGTTCTCAGACATAAAGATCAGGAG	P	CAATACGCTGCTCTCGAGCTG	P	CAATACGCTGCTCTCGAGCTG	P	CAATACGCTGCTCTCGAGCTG	P	AATTCAGTAACTGCGCTTTGGGAG	AATTCAGTAACTGCGCTTTGGAGGAG	
705	1303	AGATCATCTTCTCCAGCGCTAAC	P	CAAGGAAATTTCAATGCTGCTTG	P	CAAGGAAATTTCAATGCTGCTTG	P	CAAGGAAATTTCAATGCTGCTTG	P	AAAAATAGTTAATCTGTTTCACTTC	AAAAATAGTTAATCTGTTTCACTTC	
706	1304	AGATGACCCCTATGTTAGCT	P	AGGTAATCAATCTGTGTGGTG	P	AGGTAATCAATCTGTGTGGTG	P	AGGTAATCAATCTGTGTGGTG	P	AAATAGTAACTAAACAAAATAACATA	AAATAGTAACTAAACAAAATAACATA	
707	1305	CGTTTGTGTTTATTTTAAAGACATCA	P	GTAATTCAGCACTTTAGGACTCC	P	GTAATTCAGCACTTTAGGACTCC	P	GTAATTCAGCACTTTAGGACTCC	P	GATGCCAGCTAATTTCTCTTTT	GATGCCAGCTAATTTCTCTTTT	
708	1306	TGTAGCTGGGACTCAGGGAT	P	AAITTAACAGCTCGAAACACAGATT	P	AAITTAACAGCTCGAAACACAGATT	P	AAITTAACAGCTCGAAACACAGATT	P	AGAAAGXTGATGATTCGAGGCTATCTGT	AGAAAGXTGATGATTCGAGGCTATCTGT	
709	1307	TGGCTTAGCCTTACTCTCTG	P	TCTGCTTGACTCTGAAATCAGATT	P	TCTGCTTGACTCTGAAATCAGATT	P	TCTGCTTGACTCTGAAATCAGATT	P	ATCACAATCTTCTGCTACTATGCTATAA	ATCACAATCTTCTGCTACTATGCTATAA	
710	1308	AGGTGGCTCTATCTCAGAGGGA	P	AGGTTTCAAGTCTGATGATGAACAT	P	AGGTTTCAAGTCTGATGATGAACAT	P	AGGTTTCAAGTCTGATGATGAACAT	P	CTATGCCAATTCGACATGACATGACTAGAG	CTATGCCAATTCGACATGACATGACTAGAG	
711	1309	GGGCTCAGACATATGATGATGAACAT	P	ACGTTTCAAGTCTGATGATGAACAT	P	ACGTTTCAAGTCTGATGATGAACAT	P	ACGTTTCAAGTCTGATGATGAACAT	P	AGCCAGAGGCGAAXACTGTCTATGTCG	AGCCAGAGGCGAAXACTGTCTATGTCG	
712	1310	TGCTTCCGACAGGAGC	P	CNAGGTCNAGGATCAGGAGC	P	CNAGGTCNAGGATCAGGAGC	P	CNAGGTCNAGGATCAGGAGC	P	ATCACAATCTTCTGCTACTATGCTATAA	ATCACAATCTTCTGCTACTATGCTATAA	
713	1311	GCACGCGAAGTCTGAAGTTT	P	TGTAGTTCTGAAGTGTGGGACTTTAAA	P	TGTAGTTCTGAAGTGTGGGACTTTAAA	P	TGTAGTTCTGAAGTGTGGGACTTTAAA	P	CAACAAAATTGGTTCNAAGATGAAATAT	CAACAAAATTGGTTCNAAGATGAAATAT	
714	1312	AAATCTCCAGACACAGAGGAGC	P	AGAAATGAAGTGAACAAAAGAAATTGCG	P	AGAAATGAAGTGAACAAAAGAAATTGCG	P	AGAAATGAAGTGAACAAAAGAAATTGCG	P	TATAGACTCGATGACAGCACTACGCGCTT	TATAGACTCGATGACAGCACTACGCGCTT	
715	1313	GTACAGACATAATGGACAGCTGAAC	P	AGGAAATGAGTGTACCATCTTTTATGAC	P	AGGAAATGAGTGTACCATCTTTTATGAC	P	AGGAAATGAGTGTACCATCTTTTATGAC	P	TGTAAATTAATATCTCAAAAAGAGATG	TGTAAATTAATATCTCAAAAAGAGATG	
716	1314	GCACATCTCCGCTCATTTG	P	GGCACCTGTAAATCCCACTTAC	P	GGCACCTGTAAATCCCACTTAC	P	GGCACCTGTAAATCCCACTTAC	P	TACTCGGACGACGACGACGACAGAA	TACTCGGACGACGACGACGACAGAA	
717	1315	AAATTACTCAACATCCTGACTTTTAAAGATAC	P	CATGTTAGCAGAGATGGTCTG	P	CATGTTAGCAGAGATGGTCTG	P	CATGTTAGCAGAGATGGTCTG	P	GATCTCTGTGACTGCTGTGTCACCCAC	GATCTCTGTGACTGCTGTGTCACCCAC	
718	1316	TCAGACAGGGAGTGGT	P	AGGACAGCACTGGCACTTCC	P	AGGACAGCACTGGCACTTCC	P	AGGACAGCACTGGCACTTCC	P	TTTCAATCTGACAGACGCCCTCCCTCCA	TTTCAATCTGACAGACGCCCTCCCTCCA	
719	1317	TCAGGGTCTGGGTCTCC	P	CATGCTACACAGGCCCCA	P	CATGCTACACAGGCCCCA	P	CATGCTACACAGGCCCCA	P	CTTGTCTCACCCGACCCACXCTCAGAG	CTTGTCTCACCCGACCCACXCTCAGAG	
720	1318	TTGAGGCGCAGAGTTTGTGA	P	CNAGTACTAGCAGCTACAGATGTTGTC	P	CNAGTACTAGCAGCTACAGATGTTGTC	P	CNAGTACTAGCAGCTACAGATGTTGTC	P	TAGCCGACCTTATCTCTCAAAAATAAT	TAGCCGACCTTATCTCTCAAAAATAAT	
721	1319	TTGAGAGGGCCAAAGCATAT	P	CATGATTTCAAGGGGGAAGG	P	CATGATTTCAAGGGGGAAGG	P	CATGATTTCAAGGGGGAAGG	P	AGGAACTGTGCAGCAGTCTXTAATAAG	AGGAACTGTGCAGCAGTCTXTAATAAG	
722	1320	TTTTGTGACGAGTTTAAACAAAAGTA	P	ATTACAGGCTTGAGCCACAA	P	ATTACAGGCTTGAGCCACAA	P	ATTACAGGCTTGAGCCACAA	P	CACCTGGGCTTCCATGTTGTCATAT	CACCTGGGCTTCCATGTTGTCATAT	
723	1321	CTGGCGCTCTCTCTGCTC	P	CCTCCAGCTGCTGAGCCATAGG	P	CCTCCAGCTGCTGAGCCATAGG	P	CCTCCAGCTGCTGAGCCATAGG	P	CTTCAACCAATTCCTACTTCTTGGTG	CTTCAACCAATTCCTACTTCTTGGTG	
724	1322	TGTTATTCGATAAAGACTAGTTATGCA	P	CNAGAGGTTTATGATGCCATTAAGCTGA	P	CNAGAGGTTTATGATGCCATTAAGCTGA	P	CNAGAGGTTTATGATGCCATTAAGCTGA	P	GAAATGAATTAATTCGGCCAGTGTGAG	GAAATGAATTAATTCGGCCAGTGTGAG	
725	1323	ATGACAGACTATGACATCATTAAG	P	GTATGTCCCACTTATTCAGAGATGCA	P	GTATGTCCCACTTATTCAGAGATGCA	P	GTATGTCCCACTTATTCAGAGATGCA	P	GTACAAATTCAGGTTCTCTCTATG	GTACAAATTCAGGTTCTCTCTATG	
726	1324	TTCAACAGCTCTCAGGACG	P	CATATGCAAGCCCTCTGCC	P	CATATGCAAGCCCTCTGCC	P	CATATGCAAGCCCTCTGCC	P	ACAGTATCTGTGTCGAAATGTGCAACA	ACAGTATCTGTGTCGAAATGTGCAACA	
727	1325	ATCTCTCTGCTCTCAGCCTC	P	CNAGGTCNAGATTCGAGGTTTG	P	CNAGGTCNAGATTCGAGGTTTG	P	CNAGGTCNAGATTCGAGGTTTG	P	AGTGAGACTCTGTCTCTCAAAAATAA	AGTGAGACTCTGTCTCTCAAAAATAA	
728	1326	AGATTTGTTTCTAAGCTATTCCTAGATCTGT	P	CTGTGAGGATTAATAATAGTGAATGCA	P	CTGTGAGGATTAATAATAGTGAATGCA	P	CTGTGAGGATTAATAATAGTGAATGCA	P	ATTTTTTCTCGGCTGCGCATACGGAAAT	ATTTTTTCTCGGCTGCGCATACGGAAAT	
729	1327	TATAAAGGCTTATTTCAAGTTAGACAGTGTTC	P	CTTTTATTAAGTTTATGGCTGAGACAAAGG	P	CTTTTATTAAGTTTATGGCTGAGACAAAGG	P	CTTTTATTAAGTTTATGGCTGAGACAAAGG	P	GGTAAAGGAAATGATCTACAGGCT	GGTAAAGGAAATGATCTACAGGCT	
730	1328	AACTGCTCCTCTCTCTCAAC	P	CTGGCTCTCCCATCTTCTAAG	P	CTGGCTCTCCCATCTTCTAAG	P	CTGGCTCTCCCATCTTCTAAG	P	AGAAATCCTTATCAATXTATCTGT	AGAAATCCTTATCAATXTATCTGT	
731	1331	ATCTGACCTTATTTCTGTTCAAAACC	P	CTTTTATTAAGTTTATGGCTGAGACAAAGG	P	CTTTTATTAAGTTTATGGCTGAGACAAAGG	P	CTTTTATTAAGTTTATGGCTGAGACAAAGG	P	TGTAACTCTAAATAAAACTCTCGGCTC	TGTAACTCTAAATAAAACTCTCGGCTC	
732	1332	ATCATCCAGGGGTGGG	P	CCTTACCTCTCTCAGATAGATATGCTTAC	P	CCTTACCTCTCTCAGATAGATATGCTTAC	P	CCTTACCTCTCTCAGATAGATATGCTTAC	P	AGAAATATGTTACGCTGAGCTGGGAA	AGAAATATGTTACGCTGAGCTGGGAA	
733	1333	ATGACAGTTTGCATGATTAAGAAATTC	P	CTCCAGAAATCGCTTCTG	P	CTCCAGAAATCGCTTCTG	P	CTCCAGAAATCGCTTCTG	P	GGCAGGATTTGAAAGCCCTAACTCTGA	GGCAGGATTTGAAAGCCCTAACTCTGA	
734	1334	CCTAAGCCACTTTGGCAT	P	ATAGAGGCTCTCTCTGCTTTACC	P	ATAGAGGCTCTCTCTGCTTTACC	P	ATAGAGGCTCTCTCTGCTTTACC	P	TATACATTTCTATCTTAGTCACTTTT	TATACATTTCTATCTTAGTCACTTTT	
735	1335	TCATGGGAGCTGTGTGTGG	P	CTTCCGCTCATGAACCTTTGGC	P	CTTCCGCTCATGAACCTTTGGC	P	CTTCCGCTCATGAACCTTTGGC	P	GGCCXCAAGTCAGGCTTCAATGAGGAGG	GGCCXCAAGTCAGGCTTCAATGAGGAGG	

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	Modified GBA primer	3	Flanking sequence	4
785	1393	CTAGCAGGCGCCAGAACCTC			GCATTGATTAATAGGAATACAGATT		P	CAAGXCTTAAGTACATCTAAGTGA	AGACTTAAGTACATCTAAGTGA	AGACTTAAGTACATCTAAGTGA
786	1396	TCCTCCATGCCGACAC			CATGAGGAGCTTACTGTCTGAG		P	TCATGCTTTCAGGAGCTTACTGTG	TCATGCTTTCAGGAGCTTACTGTG	TCATGCTTTCAGGAGCTTACTGTG
787	1397	CAAGTCTCTGAAACCTGGAT			AAATGTGAGTAACTGATCTTAAGATGTGACT		P	GTGATGAGTAACTGATCTTAAGAT	GTGATGAGTAACTGATCTTAAGAT	GTGATGAGTAACTGATCTTAAGAT
788	1398	AAATTTGAACTGACGACCTG			CTTTTCTTACTAGTATGCGCCCTCA		P	ATTACTTAACTGATCTTAAGAT	ATTACTTAACTGATCTTAAGAT	ATTACTTAACTGATCTTAAGAT
789	1399	TACTAGTCTCAGAGTATTTGTGTTGAAAG			TGTTGGCCCATACCTGCTAA		P	TGAATTGATTTGGGATTAATGAAA	TGAATTGATTTGGGATTAATGAAA	TGAATTGATTTGGGATTAATGAAA
790	1400	TTTCATGCTGTGCTCGAG			GAITTTCTTCTTACACAGAGCTCACTG		P	ACCCATCCCATTTGGCTCTTCTACA	ACCCATCCCATTTGGCTCTTCTACA	ACCCATCCCATTTGGCTCTTCTACA
791	1401	CTGAGATTTATGATATTTGATGAAAG			TGGCTTACAGCTCAAGAGCTAGG		P	GGAGAAATATACCTTTTACAGTTA	GGAGAAATATACCTTTTACAGTTA	GGAGAAATATACCTTTTACAGTTA
792	1402	AATATATGCTGTGATGAGCTTTAAATACC			ATGAAATGCGCTCTGCTGG		P	GCCAAACAGCTTCTGCACTTAAG	GCCAAACAGCTTCTGCACTTAAG	GCCAAACAGCTTCTGCACTTAAG
793	1403	GTTTTATAGATTTGGGTAGATAAGG			CTTCTCTGCGCCATCT		P	TGTGACCCCACTCTGCGCCACAG	TGTGACCCCACTCTGCGCCACAG	TGTGACCCCACTCTGCGCCACAG
794	1404	TAGGCTCTGCACTTAGACTCT			CAGTGTGAGATGAGCAGG		P	TGATGCTCATCAGCTCTTGGCCCA	TGATGCTCATCAGCTCTTGGCCCA	TGATGCTCATCAGCTCTTGGCCCA
795	1405	ACCGGAGGTGAAGGCG			CAGKCTGGGAGCTGGG		P	AGCCCGCTCCGCTCTTGAATCT	AGCCCGCTCCGCTCTTGAATCT	AGCCCGCTCCGCTCTTGAATCT
796	1406	TTTQAATCTTCCGACAC			GGCTTCCCAATTTCTCTGTG		P	GTCCGCTCCGCTCTTGGCCCA	GTCCGCTCCGCTCTTGGCCCA	GTCCGCTCCGCTCTTGGCCCA
797	1407	GCTTGGCGGAGGTCT			TTTGACAGCAAGACACTTCAATC		P	AACTTAACCCATTAATTTTGTGCT	AACTTAACCCATTAATTTTGTGCT	AACTTAACCCATTAATTTTGTGCT
798	1408	ATGAGTGTGCTGATTTCAATGGTTA			CTTTGTGCTGTGACACACTTCTTG		P	TGAGTCAATGGAATCTTGGAGGCT	TGAGTCAATGGAATCTTGGAGGCT	TGAGTCAATGGAATCTTGGAGGCT
799	1409	AGAGATTTGCTGAGGTTAACTGT			CATGATCTTCCATCTCAGG		P	CATTTAAACXAGAGGAGTGGCTCA	CATTTAAACXAGAGGAGTGGCTCA	CATTTAAACXAGAGGAGTGGCTCA
800	1410	AGTGTGCTGCTGCTGCTGCTGCT			GAACATGCTGCTGTGCTGCTGCT		P	AGGAGTGTGCTGCTGCTGCTGCT	AGGAGTGTGCTGCTGCTGCTGCT	AGGAGTGTGCTGCTGCTGCTGCT
801	1411	TAACTGATTAATACCACTCACTTAGG			CATTTTGGGCACTGCT		P	TGCTGCAAGGCTCTGCACTTAGG	TGCTGCAAGGCTCTGCACTTAGG	TGCTGCAAGGCTCTGCACTTAGG
802	1412	CTTGTGCTGCTGCTGCTGCTGCT			ACAGCAAGGAGGAGGAGGAGG		P	GTGCTGCTGCTGCTGCTGCTGCT	GTGCTGCTGCTGCTGCTGCTGCT	GTGCTGCTGCTGCTGCTGCTGCT
803	1413	CAGTTATACCTTTTAACTCTACTGGG			CACTTCCGCTGCTGCTGCTGCT		P	GAATACAGCTTCTGCACTTAGG	GAATACAGCTTCTGCACTTAGG	GAATACAGCTTCTGCACTTAGG
804	1414	CATATGCTAGTGTGATGATGATGAT			AACTCTGCTTGGGCACTGTTAA		P	TGTTAATTAACATCTTACAGCTCA	TGTTAATTAACATCTTACAGCTCA	TGTTAATTAACATCTTACAGCTCA
805	1415	AAACAGCTGCTGCTGCTGCTGCT			TTTGAACCTTCACTGCTGCTGCT		P	ACATCAGGAGGCTGCTGCTGCTGCT	ACATCAGGAGGCTGCTGCTGCTGCT	ACATCAGGAGGCTGCTGCTGCTGCT
806	1416	AACTAGTGTGCTGCTGCTGCTGCT			AGAGAGGAGGAGGAGGAGGAGG		P	AACTCTTAACTTCACTGCTGCTGCT	AACTCTTAACTTCACTGCTGCTGCT	AACTCTTAACTTCACTGCTGCTGCT
807	1417	CAATATGCTGCTGCTGCTGCTGCT			TCAGCTGTAGTGTGCTGCTGCTGCT		P	CGCTTAATTTCACTGCTGCTGCTGCT	CGCTTAATTTCACTGCTGCTGCTGCT	CGCTTAATTTCACTGCTGCTGCTGCT
808	1418	GCTGACTTTCAGTGGGAGG			TCCTGCTTATCTGCTGCTGCTGCT		P	ATTGATTAAXAAATCTTATAGTAC	ATTGATTAAXAAATCTTATAGTAC	ATTGATTAAXAAATCTTATAGTAC
809	1419	AATGCAATTTGCTGCTGCTGCTGCT			ACTTCCAGCTTGAATATAGTGTGCT		P	AGTCAAGAACTTCACTGCTGCTGCT	AGTCAAGAACTTCACTGCTGCTGCT	AGTCAAGAACTTCACTGCTGCTGCT
810	1420	AGAACTTACTAGACAGAGAGATTTGCTG			CCCTCCTCCAGAGGCTGCTGCTGCT		P	CCTCAGGAGTGTAGATGCTGCTGCT	CCTCAGGAGTGTAGATGCTGCTGCT	CCTCAGGAGTGTAGATGCTGCTGCT
811	1421	CACCAAGATGATGATGATGATGATGAT			TGGGCTTATATAGATGATGATGATGAT		P	CTGTTGCTCCAGGCTGCTGCTGCTGCT	CTGTTGCTCCAGGCTGCTGCTGCTGCT	CTGTTGCTCCAGGCTGCTGCTGCTGCT
812	1422	TTAGAGGAGTCTGATGATGATGATGAT			TGACAGGCTGAGAGGCAACA		P	AGAAATCTGACAGAGTCTTAACTG	AGAAATCTGACAGAGTCTTAACTG	AGAAATCTGACAGAGTCTTAACTG
813	1423	TGAGCAAAAGTGTGATGATGATGATGAT			CACCTGCTGCTGCTGCTGCTGCTGCT		P	AGGGGCTTGTGAGGCTGCTGCTGCTGCT	AGGGGCTTGTGAGGCTGCTGCTGCTGCT	AGGGGCTTGTGAGGCTGCTGCTGCTGCT
814	1424	ATCTTACAAAGAGCAAAATATAGCTG			CCTCAGGCTGATTTGCTGCTGCTGCT		P	TCCAGCTGCTGAGGCTGCTGCTGCTGCT	TCCAGCTGCTGAGGCTGCTGCTGCTGCT	TCCAGCTGCTGAGGCTGCTGCTGCTGCT
815	1425	TACTTATTTCTATCCAGGCTGCTG			CAGTCTATTTTCCGCTGCTGCTGCT		P	GGGGATCTGCGGCTGCTGCTGCTGCT	GGGGATCTGCGGCTGCTGCTGCTGCT	GGGGATCTGCGGCTGCTGCTGCTGCT
816	1426	TCAACTGCTATTTGCTGCTGCTGCT			CAGAACTGCTGCTGCTGCTGCTGCT		P	GTCTCTCTCTCTCTCTCTCTCTCTCT	GTCTCTCTCTCTCTCTCTCTCTCTCT	GTCTCTCTCTCTCTCTCTCTCTCTCT
817	1427	CTAAAGATGCTGCTGCTGCTGCTGCTGCT			TGTACAGATGCTTATCAGAGATGCTGCT		P	TCAGGCTTATGCTGCTGCTGCTGCTGCT	TCAGGCTTATGCTGCTGCTGCTGCTGCT	TCAGGCTTATGCTGCTGCTGCTGCTGCT
818	1428	TAGCAAGATGCTGCTGCTGCTGCTGCTGCT			TCAAATATTAATGCAAGATGCAACA		P	TTACTCAGATATGCTGCTGCTGCTGCT	TTACTCAGATATGCTGCTGCTGCTGCT	TTACTCAGATATGCTGCTGCTGCTGCT
819	1429	CGAGTGTGCTGCTGCTGCTGCTGCTGCT			TCCTTGTGCTGCTGCTGCTGCTGCT		P	ATATGCTTCTTCTTCTGCTGCTGCTGCT	ATATGCTTCTTCTTCTGCTGCTGCTGCT	ATATGCTTCTTCTTCTGCTGCTGCTGCT
820	1430	TTTCTCTCTCTTAAATGCTGCTGCTGCT			ATAACTCTCAGAAATAGAGAAATGCTG		P	GTGACXGTCTGCTGCTGCTGCTGCTGCT	GTGACXGTCTGCTGCTGCTGCTGCTGCT	GTGACXGTCTGCTGCTGCTGCTGCTGCT
821	1431	ACGAGATCTCAATATCTGCTGCTGCTGCT			CTTCAGGCTGCTGCTGCTGCTGCTGCT		P	TTGGCTCTGCTGCTGCTGCTGCTGCTGCT	TTGGCTCTGCTGCTGCTGCTGCTGCTGCT	TTGGCTCTGCTGCTGCTGCTGCTGCTGCT
822	1432	AAAGGAGAGGAGGAGGAGGAGGAGGAGG			GCAGAAATGAGGCTGCTGCTGCTGCTGCT		P	GTCTGCTGCTGCTGCTGCTGCTGCTGCT	GTCTGCTGCTGCTGCTGCTGCTGCTGCT	GTCTGCTGCTGCTGCTGCTGCTGCTGCT
823	1433	ATCTTCTGAGGCTGCTGCTGCTGCTGCTGCT			CCTTAGTCTTCTGCTGCTGCTGCTGCTGCT		P	CACTGCTTCTGCTGCTGCTGCTGCTGCT	CACTGCTTCTGCTGCTGCTGCTGCTGCT	CACTGCTTCTGCTGCTGCTGCTGCTGCT
824	1434	TACAGATCTCTATCCAGGAGG			CAAAATGCTTCTTCCGCTGCTGCTGCTGCT		P	AGGCTGCTGCTGCTGCTGCTGCTGCTGCT	AGGCTGCTGCTGCTGCTGCTGCTGCTGCT	AGGCTGCTGCTGCTGCTGCTGCTGCTGCT
825	1435	TACTTAAATACAAAGATGCTGCTGCTGCT			CTCGCTCTCCAGGCTGCTGCTGCTGCTGCT		P	CGAGTCTGCTGCTGCTGCTGCTGCTGCT	CGAGTCTGCTGCTGCTGCTGCTGCTGCT	CGAGTCTGCTGCTGCTGCTGCTGCTGCT
826	1436	TACTTAAATACAAAGATGCTGCTGCTGCT			AGAGCTTAAAGATGCTGCTGCTGCTGCTGCT		P	TTCCAAAGAAATAGAGGAGGATTA	TTCCAAAGAAATAGAGGAGGATTA	TTCCAAAGAAATAGAGGAGGATTA
827	1437	TCTACAGAGGAGGAGGAGGAGGAGGAGG			GGATATCTTCTGCTGCTGCTGCTGCTGCTGCT		P	TTCCCTTAAAGATGCTGCTGCTGCTGCTGCT	TTCCCTTAAAGATGCTGCTGCTGCTGCTGCT	TTCCCTTAAAGATGCTGCTGCTGCTGCTGCT
828	1438	AAATATGCTGCTGCTGCTGCTGCTGCTGCT			CAAAATGCTGCTGCTGCTGCTGCTGCTGCT		P	TTGCAATCTAGTCTGCTGCTGCTGCTGCT	TTGCAATCTAGTCTGCTGCTGCTGCTGCT	TTGCAATCTAGTCTGCTGCTGCTGCTGCT
829	1439	AAATATGCTGCTGCTGCTGCTGCTGCTGCT			CAAAATGCTGCTGCTGCTGCTGCTGCTGCT		P	TTGCAATCTAGTCTGCTGCTGCTGCTGCT	TTGCAATCTAGTCTGCTGCTGCTGCTGCT	TTGCAATCTAGTCTGCTGCTGCTGCTGCT
830	1440	CATGAGTGTGCTGCTGCTGCTGCTGCTGCT			CAAAATGCTGCTGCTGCTGCTGCTGCTGCT		P	TTGCAATCTAGTCTGCTGCTGCTGCTGCT	TTGCAATCTAGTCTGCTGCTGCTGCTGCT	TTGCAATCTAGTCTGCTGCTGCTGCTGCT
831	1441	AGAAAGCTGCTGCTGCTGCTGCTGCTGCTGCT			CTCGAGGCTGCTGCTGCTGCTGCTGCTGCT		P	GGTGAAGCTTATGCTGCTGCTGCTGCTGCT	GGTGAAGCTTATGCTGCTGCTGCTGCTGCT	GGTGAAGCTTATGCTGCTGCTGCTGCTGCT
832	1442	TAACTAAACAAATATCTGCTGCTGCTGCT			GGCCCTCTTAAACAAATATCTGCTGCTGCT		P	CCCAAGCTGCTGCTGCTGCTGCTGCTGCT	CCCAAGCTGCTGCTGCTGCTGCTGCTGCT	CCCAAGCTGCTGCTGCTGCTGCTGCTGCT
833	1443	ATTATTAATATCCCAAGGAGGAGGAGGAGG			CATTAACAGAGCTGCTGCTGCTGCTGCTGCT		P	CCAGGCTGCTGCTGCTGCTGCTGCTGCTGCT	CCAGGCTGCTGCTGCTGCTGCTGCTGCTGCT	CCAGGCTGCTGCTGCTGCTGCTGCTGCTGCT
834	1444	CTTGGCTAGTGTGCTGCTGCTGCTGCTGCTGCT			TTAATGCTACAGAAATAGAGTCAACACT		P	GAAGTCAACAGTACAAAGAGGAGGAGGAGG	GAAGTCAACAGTACAAAGAGGAGGAGGAGGAGG	GAAGTCAACAGTACAAAGAGGAGGAGGAGGAGGAGG

[illegible]

Row#	REF#	Upper PCR primer	1	2	Lower PCR primer	3	4	Flanking sequence
883	1501	CTGTGTTGGCTCACAGCTG	P	CTAATTTTGTATTTTATAGTAGAGCGGG	P	ACTCTCACCTCAAGTGATACACCC	ATCCGAGCACTTTGTGAGGCGGAGGGGTGTATCACTTGAAGTCAGGAGT	
884	1502	AAAGCAAAAACACAAAGCATC	P	CAGAGGGCTTTGGATGCA	P	CAGAAAGGTCAATGCCTCACTCAGAG	CAAAGGTCAAGTGCCTCACTCACTAGGGCCACAGACTAGCATCAGAGTCTGGG	
885	1503	GCTTTAAACAAATACAAAACAAAAG	P	ACGCTAACTGGGAGTCAAGCTG	P	CAATGTTACAAAGCTCAAGCATGGTA	CTCTTGGAGCTGCTCTAAATTCATATACCACTGCTGAGCTGTGTATCACTTG	
886	1504	CTGCGCTCAGACACACAGC	P	GTGCGTTGGGATTTGTG	P	GGCCACGGCCACACXCTCCACAGCC	CACAGACGAACATCTGTAAAAACTCGCTGGGTGGAGCTGCTGGCGGTGGCC	
887	1505	ATTAGGACTATTCCGCTGGCCAA	P	CAATATATATAGAGAGCTCCGTAGTGGG	P	ATATCTCAGAGACACATTTCAAGGT	GCTAAACACTTTGTGCTTTGAGAGTTTGACTTTTGAANAATGTGCTCAGAGATAT	
888	1506	GCAGTGTATTTGTATGGGTCTATTTC	P	TCAGGTGACGTGACATATATCAATTTTC	P	TAAACAGCTGTAGTXTTGGAGATAA	TTCTGTTTAGTGAATCTATCTGTCTATATATTTCTTCCATATACATACATGTTT	
889	1507	GAGTCTTCCCTGTGGTGCAGT	P	AGTTGAGCGCTGATAGGTATTTATGGAT	P	TCGGGTTTGGACTACTCATCTCATTTG	TCGGGTTTGGACTACTCATCTCATCTTGTGTTGATCTTCACTTCACTATMAA	
890	1508	CGTTCCGACCTTTTGNACACA	P	TGAGCTAGAAAAGTAATTTGCTGCTC	P	GTCAGTCCCTGTAGCAGAATTTGTGAC	ACAGCTTTTGGATATAAAATTTATAAACCTTTCACACAACTTTCACACAGACTG	
891	1509	CCGAGGTGCTCTAAGGCA	P	GATATGTGCTTTTCCGAAAACAA	P	CTTGXGCAAGTCAAGTAACCTGAGTGT	GCCCGTTTACGAGAGAGCGAAATGACACTGAGGTTTACTGACTTTCGCCA	
892	1510	CAATGCAATTCAGGCTTTGAGG	P	TCTGAGTTTGGCAAAAGTAGCC	P	AGAGCTTTCAATCATGCGAAGACATC	AGAGCTTTCAATGCGCAAGACATGCACTCTTTTTCAGCAAAATTCGAAAGT	
893	1511	CAAGTGGCTCATCTGTGG	P	GTCGATTTCTGTGTTTGGGG	P	GACAGAGTTCTGAGAGTAAATGAC	CAGCGGCTCATTTCTAATGTTATGTGATGATTAAGTCTTAGAAAATCTGTGT	
894	1512	CGAGTCTGACAGATATGATGACATAT	P	AGTTAGGTTCTATTTCTTTCTTTTCT	P	TTCTTTTTCGACTTTTATCATTTGTA	GACTGGGAAATCATGTTAAGTTTCTTCTGATCAATATGATAAAAAGTCAAAAAGAA	
895	1513	ATTACAGACAGAGAGGCC	P	CNATGCTGAGAGGGGCTCC	P	TCAGGCTNTACAAACGAAACCCAC	TGGGCTGAGGAGAGCTCGAACAGCTGGGTTCTGGTTGTATGACCTTCA	
896	1514	TTTATTTCCATCTTCTTATTCATACTGG	P	ATTGAGCCGACGAGAGC	P	ACGAGACAGAACTTAATCCXGCAAG	CGCGACCAATGAGCGCTAGAACAGCTGAGAACGCTGGGAGTTAGGTTCTCTCT	
897	1515	ATGCAACACATCCCTCTCTCA	P	GTACAGCCCTCGATTGTTTCTTC	P	CCCGCTCACCCACTCATGCTGCTC	CCCGCTCACCCACTCATGCTGCTCATCCCTCATCTTATCTGGGAGTCTCT	
898	1516	GAGTAGTAGAGAGTTTAGAGAGCTACAAAC	P	CTACAGCCCTCGATTGTTTCTTC	P	AGTCTGGGATTTACAGAGCTGAGC	GCTCTGTACTCATTTGATTTTTCAGAGACCTCTGAGGCTGGGAGCTTTTG	
899	1517	TTTTCCAGTCTGAAACATAAAGGA	P	CCGNCCTTTGGCTCCCA	P	AAAGTCTGGGATTTACAGAGCTGAGC	AAATGCTATTCGACCGGGCGAGTGGCTCAAGCTGCTTAATCCGAGCACTT	
900	1518	CAAAATAGTCTTAAATCAAGCATGA	P	CGCTCCGCTCCCAAG	P	AGTCTGAAATTTACAGAGCTGAGCCAC	TAAAGCTGCTTCTCGCGCGCGGGCTGGCTCAAGCTGCTTAATTTTCAGCA	
901	1519	TCGGTGCAAGACATACATTAAG	P	GTTCGTGTCGAAAAGAAAGTAAATGAGA	P	AAATCAGACAGACAGCCGCAAGCT	CCCGCATGTGACCTCCCGAGCGCGAGTGTGGGCTCTCTCTGTCTATTT	
902	1520	GAAGGCTTCCCTGAGCGTG	P	GTGCCACTTTGAAAGGTTGAAAC	P	TGTCCGCTCTCTCTTCCGCAAAATG	CCCTCATTTGTGCTGTGCAAAATGTGGCATTTTGTCTGAAAGAGAGACGGCA	
903	1522	CTGTTTTCAGGATTAATCAGCAG	P	TCCTGGGTCAAGAAAAGTCTG	P	CTTCCAAAATGTGTGACGTAAATGTC	CGTTATTTAAGAAATGTGTAAGAGACACATCTTACTGACACATTTTTCGAAC	
904	1523	CGCATCATTCGCCCTTCC	P	CTTACTTTCTCCATTTACCTTAGAGGGA	P	AATCAGACTTACATTAAGACAGACTC	ATTACAGACTTACATCAAGTTCAACGAGCTCTCTGTGTAATGTAGTGAT	
905	1524	AAATATTCAGCGCTGCC	P	GGCCTGAGGAGGCAAG	P	AGGCACTCTFAGTGGCAACATCTCA	TCCTATGGGTTAGAAAAGGCTATCTGTAGATGTTTCCCACTATAGAGAGTGC	
906	1525	TAATCTCTTTCTCGCCGACTG	P	CATGTTCTTTTGTGATGATGGC	P	CAAXATCTCATACACATCTGCTC	TGGGTCGACTTTGGCCAAATGCAATGGGAGCAGATGTTGTATGATACAACTCT	
907	1527	AAATACTGATTTTAAATGGTTTGTGATGC	P	CTTCTGTTTCAACGCTGTACACATATG	P	CAAGATCMAAAATCTCACAGCTGTA	CCAGGATCMAAAATCTCACAGCTGAGCTGCAATGGCAGCGACAGATTTAGAA	
908	1528	CCACACTCGGACGCCAA	P	CTACAGCCCGCCCTCGT	P	CTCCAGGGATXTCGCCAGCTGAGCG	CCAGGGATCTCCAGCTGAGAGAGGCAAGCGGAGCTCGCGGCTCACAGCT	
909	1529	TAAACAAAGATGCAATTTATACAGG	P	CTCCCTGTGCCCCAAGT	P	CGAAGGGGGGGGAAAATCTCAAGGTTT	CGAAGGGGGGGGAAAATCTCAAGGTTTCAAGAGGGGGGCTTTCCMACT	
910	1530	AAACCACATCTATGACATCATAGG	P	GTCTTTTGACACCTATAGGCTTAAGGG	P	ATGTAAGGGTTTTTTCAGCTCTAT	ATGTAAGGGTTTTTTCAGCTCTTATGTGCTGACTCTCATATAGATGTCACTGC	
911	1531	AGACTCGCTGGGCGTAAATTT	P	GAACTTGATGTGTGTTGTGGAA	P	TAAGCAATATAAGAGCTAAAGGTT	ATTTATACAGGAAAATTTTAAACCGAGCTGAGGCTTTTACCTTTATTTATGCTCTA	
912	1532	CGAAAGAGATGGGTGGGG	P	TCGAGAGGTCATGTTAATACAGAG	P	TGCACATAAAGCCAGAGCTCACTT	TGCACATAAAGCCAGAGCTCACTTCTCTTTCCGACAGCTCAGAGAAAATCCT	
913	1533	TAACCCATCTATGCAATCATATAC	P	CAGAGAGGAAAGATGCAACATTT	P	CATTCACTGGGTCCTTACAGAGAT	AACTGTAAANAATAGGACATTTACTACATCTGCTGTAGAGAGCCCATGAAATG	
914	1534	TCGTTGTGGCATAGCA	P	CACAAATGCGCAGGAGCA	P	TGCACATAAAGCCAGAGCTCACTT	TGCACATAAAGCCAGAGCTCACTTCTCTTTCCGACAGCTCAGAGAAAATCCT	
915	1535	AGCTTGACTCTATTTTAGCATTTTG	P	CTGCTATGCTCTGGCATAG	P	CTTGACTCTATTTTAGCAGTATTTG	CTTGACTCTATTTTAGCAGTATTTTGGGTGGAAATTTGGGACCCCATCTCTTC	
916	1536	TGAAAGCATACATCATATTAAGA	P	CAGTACAGCTGAGATCAACAGC	P	TTCCAGCTCCGTCTAGCCTCCCTCAT	TTCCAGCTCCGTCTAGCCTCCCTCATTTGGGTGGAAATTTGGGACCCCATCTTC	
917	1537	CTGTAGCCAAAGCAGGGGTT	P	GATTCATGATGGAATAACATCAA	P	TGCCATCTTCTGACACACCCCAACA	AGCAGCTCCGAGCTTCAACAGAGACTGTTTGGGTGGTCAAGAGATGGCA	
918	1538	AGGGGGGCTCTGCTCTCA	P	CTCCAGAGCTCTGCGGGA	P	TGAAGGACACCCCTGAGCGAAGTG	GACCCCTATAGAAGCTCAGCTTCCCGCATCTCCGCTCAGAGCTGTGCTCTTCA	
919	1539	TCTCAGGCTCAAGGGCAT	P	CACACTGAGACACTCGCTCTG	P	TTACTCTGTTTAAAGCAAGACTCT	TTACTCTGTTTAAAGCAAGACTCTGCATGCTCTATTCTCATCATGACTGAGA	
920	1541	TTCTTTAAITTCGAGAGCAATAATTCC	P	GTTTTCAGGTGATCGAGGAAA	P	ACGCTCTAAGTATTTCAAACTAGCCCA	ACGCTCTAAGTATTTCAAACTAGCCCATCTTCAAGCTGCTTACTTGGCTTGG	
921	1542	CTGGGCTCATGACCTTTTTTAA	P	CTCATGACATTTTCCCCCAT	P	ACTACCATTCACAGATATTCCGCC	GTGTGAGCTTATCCCATGCTACATGCGGGGGGATATCTGCTGAAATGCTGAGT	
922	1543	AAATGAGGAGTCAAGCTGCTCT	P	CTTTCTGGGATGGGAG	P	CTGTCGCTTCTTCTCTCATCCCAAC	CTGTCCCTTCTTCTCTCATCCCACTCTCTCAAGCCCTCTTCAAGCCACCAACAGAGC	
923	1544	TGGGTCCCTGAAATTTATATCA	P	AGTCCGCTCTCTCAATAGTTGGGT	P	CACXKATTCGCAAGCCAAAGCAGACTG	ATAAATATAATAGCTTAATATTTATCAAGTCTTGGCTTGTGGTTGGATAGTGT	
924	1545	ATCTCTACTAANAATAAANAAGTAGGACAG	P	CAATCTGGGCTCAACGCA	P	CCTCCGGGTTTAGAGCGATTCTCTCT	AGTCCAGCTACTCAGAGGCTTAGAGGAGGAGAAATCGCTCTTAAACCGGAGAG	
925	1546	ATATGGGTTTCACTTTTAAAGATJTGST	P	CTTGGCTCCAGGACAAAG	P	AAGGGCTCAGGCTGCGCCGATCTG	GTCCAGGAGCTGCGCCGATCTGCGCATCTTGGGCTTGTCCGACCATCCGAC	
926	1547	TTTAGTTTAAAGCTGAGAGAGCCC	P	TGTGCAATTCGAGGTAGTTACTGAAA	P	TACTCMAAATKCATAGTCCGACTAGT	CAGAGCATGCTGTGGCCCGGGATTAATCAGTCCGCTCATATGCAATTTTCAG	
927	1548	GTATCTAGGCTCCGAAATTCGA	P	AATGTGTTGTGTTCAAXCTACATAGT	P	AAAATGTTGTTTCAAXCTACATAGT	ATGTGTTGTGTTCAAGCTACATAGTCCGATAGAAAGCTTGTGTTATAGTAGGCC	
928	1549	GTGGTGGCTTCTGCTTAGGTC	P	CTCATGAGTGTGCTTCTGNTA	P	CTCTCTCTCCAGTGAATTTCTGNTA	CTCTCTCTCCAGTGAATTTCTCATACTTTTGAAGGAGCACTCTCGGCGCTGCTTT	
929	1550	TGCTCTCAACTTTTCCATTTTCTGC	P	TGTCAGCTCAGCTGTGAGGAAAT	P	AAATCAGGCAAGCTGGAGCCCTCAGAAAT	AAATCAGGCAAGCTGGAGCCCTCAGAAATTTCTCTTAGGCGTCTTGGGAGTGT	
930	1551	TGAGTGACCCAGATGCC	P	CGAAGTGGCTGTGGAGTAGCGG	P	GTAGTGGGTGCTTGGAGTAGACGG	GTAGTGGGTGCTTGGAGTAGCCGCAACATTTCTCGTTCAGGCGTCTGAGGCTCTAC	
931	1552	GAAITGGCTGTTTCACAGGATC	P	TGTCGTTGTTATGTAGTATCCTTTTTCC	P	CAGATCTAAACCTTTTCTXTACAAGGTTA	GATCTAAACCTTTTCTGTACAGATTTAGGTTACCAAAAGAGGAGCAATTAGAGGGA	

Row#	REF#	Upper PCR primer	1	Modified	Lower PCR primer	2	Modified GBA primer	3	Flanking sequence	4
932	1553	CCGAGGAGAGACAGTCGGA		P	TTAAACATTAAACGAGCTCCTTCCTC		AGCKCACAGAGGAGAGAGCGGACACTCA		ACTGGAGCGGCCAGAGAGCGGCTAGCTGAGTCTCGGCTTCTGCTCTGTGCG	
933	1554	TGCTTCTGCCCCACAGC			CATCACAGTTTCTGAGGCC		TATCAGAGGAGCTGGCTTTTTCACACCA		TCAGAGGAGGTGGCTTTTTCACACCGGCAATATTTTAAATCAAGAGCTGTG	
934	1555	TTGCGCTGCTGGGTGGC		P	CACACTCCCATGAGGAGT		TGACAGAGCAGTGAAGCCAAAACCCC		TCAAAACATCAGCTTTTTCGAGGGTGTGGGGTTTGGCTGACTGCTCTGTCA	
935	1556	AAGCCTCAACTATATAGTCTGCTG			GATTTCATTTCGATCTCTTTTCCTAG		GTGATATATTGAATCAAAATCAGGC		GGATATATTGAATCAAAATCAGGCCTGGCCCTTGGCCCTCATAGAGCTAG	

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which
5 is described and claimed.

We claim:

1. An oligonucleotide comprising a nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, or complementary sequences thereof, wherein n=0 through 934.

2. The oligonucleotide of claim 1, wherein X, when present in said oligonucleotide, corresponds to a single-nucleotide-spacer selected from the group consisting of anucleosidic moieties, abasic moieties, non-naturally occurring nucleotide analogs, and non-Watson/Crick base moieties.

3. An oligonucleotide comprising a nucleic acid that hybridizes with a first portion of nucleic acid selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+4, or complementary sequences thereof, wherein said first portion to which said oligonucleotide hybridizes also hybridizes with a nucleic acid selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, and wherein said oligonucleotide does not hybridize with a nucleotide base on said SEQ ID NO:4n+4, or complementary sequences thereof, corresponding to the nucleotide immediately adjacent the 3' end of said SEQ ID NO:4n+3, wherein n=0 through 934 and n is the same value for both SEQ ID NO:4n+4 and SEQ ID NO:4n+3.

4. An oligonucleotide comprising a nucleic acid that hybridizes with a first portion of nucleic acid selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+4, or complementary sequences thereof, wherein said oligonucleotide does not hybridize with a second portion of said SEQ ID NO:4n+4, or complementary sequences thereof, wherein said second portion of said

SEQ ID NO:4n+4, or complementary sequences thereof,
hybridizes with a nucleic acid selected from the group of
SEQ ID NOs consisting of SEQ ID NO:4n+3, and wherein said
second portion of said SEQ ID NO:4n+4, or complementary
5 sequences thereof, also includes a nucleotide base on
said SEQ ID NO:4n+4, or its complement thereof,
corresponding to the nucleotide immediately adjacent the
3' end of said SEQ ID NO:4n+3, wherein n=0 through 934
and n is the same value for both SEQ ID NO:4n+4 and SEQ
10 ID NO:4n+3.

5. An oligonucleotide comprising a first
portion of nucleic acid selected from the group of SEQ ID
NOs consisting of SEQ ID NO:4n+4, or complementary
sequences thereof, wherein said oligonucleotide excludes
15 a second portion of said SEQ ID NO:4n+4, or complementary
sequences thereof, wherein said second portion of said
SEQ ID NO:4n+4, or complementary sequences thereof,
hybridizes with a nucleic acid selected from the group of
SEQ ID NOs consisting of SEQ ID NO:4n+3, and wherein said
20 second portion of said SEQ ID NO:4n+4, or complementary
sequences thereof, also includes a nucleotide base on
said SEQ ID NO:4n+4, or its complement thereof,
corresponding to the nucleotide immediately adjacent the
3' end of said SEQ ID NO:4n+3, wherein n=0 through 934
25 and n is the same value for both SEQ ID NO:4n+4 and SEQ
ID NO:4n+3.

6. An oligonucleotide comprising a nucleic
acid sequence selected from the group of SEQ ID NOs
consisting of SEQ ID NO:4n+1, or complementary sequences
30 thereof, wherein n=0 through 934.

7. An oligonucleotide comprising a nucleic
acid sequence selected from the group of SEQ ID NOs

consisting of SEQ ID NO:4n+2, or complementary sequences thereof, wherein n=0 through 934.

8. A pair of oligonucleotides comprising two nucleic acids selected from the group of SEQ ID NOs
5 consisting of SEQ ID NO:4n+1 and SEQ ID NO:4n+2, or complementary sequences thereof, wherein n=0 through 934, and wherein said primers correspond to two consecutive SEQ ID NOs having the same value for n.

9. A triplet of oligonucleotides comprising a
10 nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+1, SEQ ID NO:4n+2 and SEQ ID NO:4n+3, or complementary sequences thereof, wherein n=0 through 934, and wherein said oligonucleotides correspond to three consecutive SEQ ID NOs having the
15 same value for n.

10. A kit comprising at least one oligonucleotide of claims 1, 3, 4 or 5.

11. The kit of claim 10, further comprising two amplification primers.

20 12. The kit of claim 11, wherein said primers comprise a nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+1 and SEQ ID NO:SEQ ID NO:4n+2, or complementary sequences thereof, wherein n=0 through 934, and wherein said three
25 oligonucleotides correspond to three consecutive SEQ ID Nos having the same value for n.

13. A kit comprising an oligonucleotide, wherein said oligonucleotide comprises a nucleic acid sequence selected from the group of SEQ ID NOs consisting

of SEQ ID NO:4n+3, or complementary sequences thereof, wherein $n=0$ through 934.

14. The kit of claim 13, further comprising two amplification primers.

5 15. The kit of claim 14, wherein said primers comprise a nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+1 and SEQ ID NO:4n+2, or complementary sequences thereof, wherein $n=0$ through 934, and wherein said three oligonucleotides
10 correspond to three consecutive SEQ ID NOs having the same value for n .

16. A kit comprising the two oligonucleotides of claim 8.

15 17. A kit comprising the three oligonucleotides of claim 9.

18. A method of genotyping a nucleic acid sample comprising hybridizing the oligonucleotide of claims 1, 3, 4 or 5 to the nucleic acid sequence selected from the group of SEQ ID NOs consisting of SEQ ID
20 NO:4n+4, or complementary sequences thereof.

19. The method of claim 18, wherein said oligonucleotide is employed in a primer extension reaction.

20. The method of claim 19, wherein said
25 primer extension reaction is a single-nucleotide primer extension.

21. A method of genotyping a nucleic acid sample comprising performing a primer extension reaction employing the oligonucleotide of claims 1, 3, 4 or 5.

22. The method of claim 18, wherein said
5 primer extension reaction is a single-nucleotide primer extension.

23. A method of genotyping a nucleic acid sample comprising performing a primer extension reaction employing an oligonucleotide comprising a nucleic acid,
10 or fragment thereof, selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, or complementary sequences thereof, wherein n=0 through 934.

24. The method of claim 23, wherein said
15 primer extension reaction is a single-nucleotide primer extension.

25. The method of claim 23, wherein said fragment excludes from 1 up to 10 nucleotides from the 3' end of said SEQ ID NO:4n+3.

26. A fragment of SEQ ID NO:4n+3, wherein n=0
20 through 934, wherein said fragment excludes from 1 up to 10 nucleotides from the 3' end of said SEQ ID NO:4n+3.

27. A method of genotyping a nucleic acid sample comprising:

25 a) amplifying a target nucleic acid sequence that hybridizes to an oligonucleotide selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, or complementary sequences thereof; and

- b) performing a single-nucleotide primer extension reaction employing an oligonucleotide comprising a nucleic acid selected from the group of SEQ ID NOs consisting of SEQ ID NO:4n+3, or complementary sequences thereof,

wherein $n=0$ through 934 and is the same value in both steps a and b.

28. A mixture of reagents comprising:
- (a) 10 to 200 mM buffer, having a pH ranging from 6.0 to 9.0;
 - (b) 15 to 250 mM monovalent-cation salt;
 - (c) 0.5 to 25 mM divalent-cation salt;
 - (d) 0 to 0.02% volume exclusion agent
 - (e) 0.25 to 2.5 mM of each of dATP, dCTP, dGTP and dTTP.

29. A method of amplifying a nucleic acid sample comprising the steps of:

- (a) combining two amplification oligonucleotides with said nucleic acid sample;
- (b) adding to said nucleic acid a reagent comprising:
 - (i) 10 to 200 mM buffer, having a pH ranging from 6.0 to 9.0;
 - (ii) 15 to 250 mM monovalent-cation salt;
 - (iii) 0.5 to 25 mM divalent-cation salt;
 - (iv) 0 to 0.02% volume exclusion agent
 - (v) 0.25 to 2.5 mM of each of dATP, dCTP, dGTP and dTTP.

(c) contacting said nucleic acid with a nucleic acid polymerase.

30. An oligonucleotide comprising a nucleic acid sequence selected from the group of SEQ ID NOs
5 consisting of SEQ ID NOs: 1-3740, or complementary sequences thereof.

Diagram of GBA Process

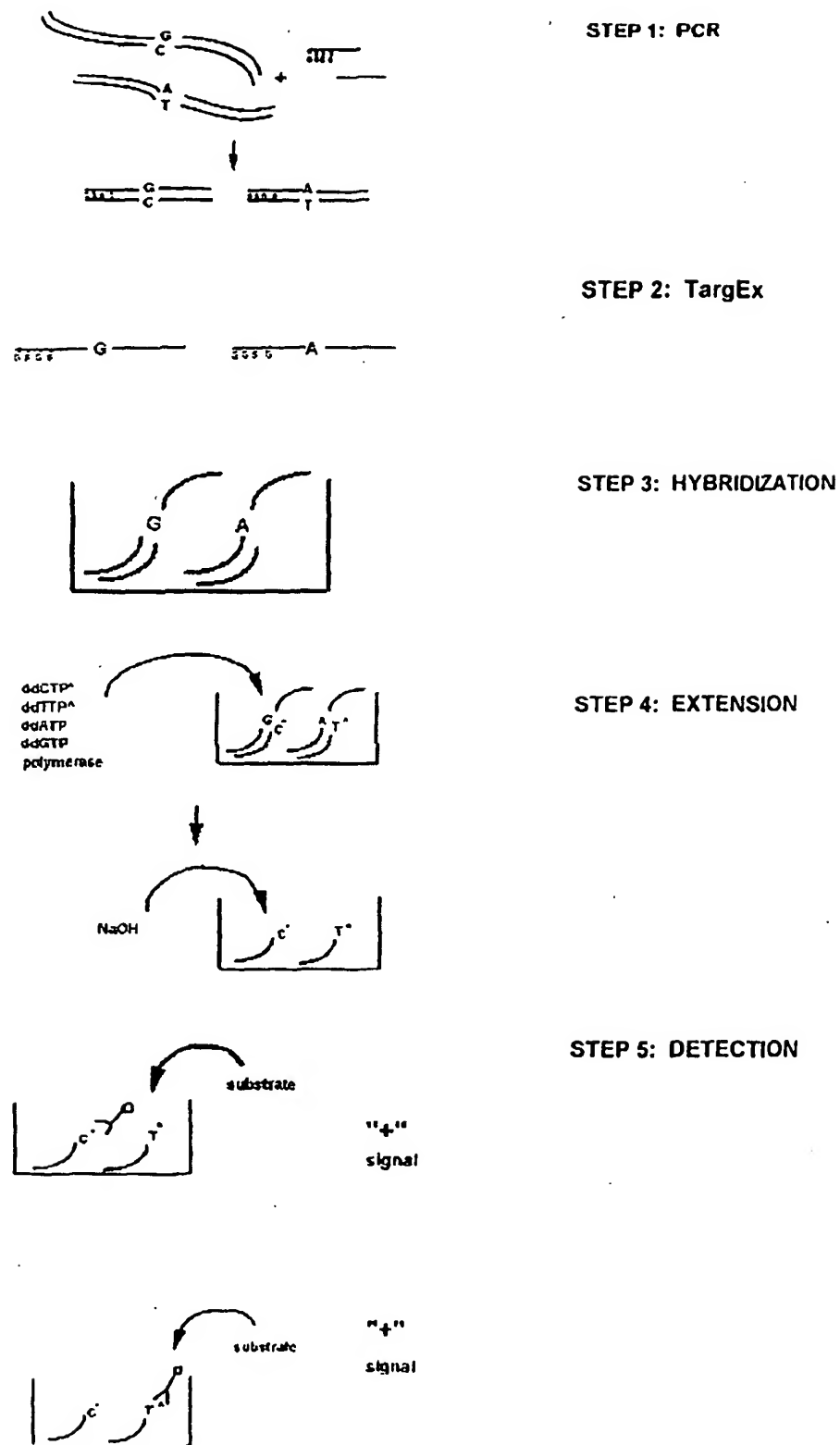


Figure 1

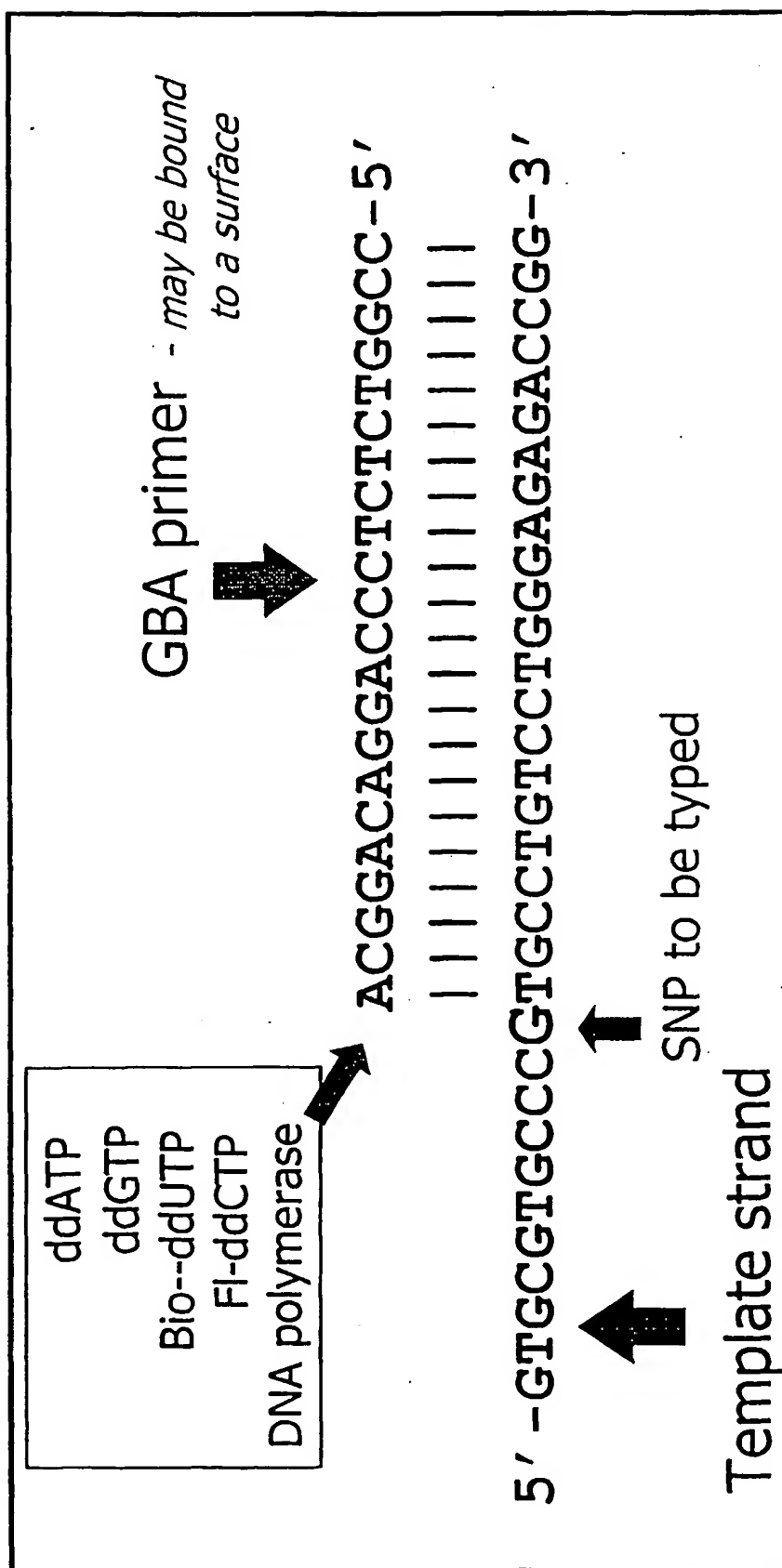


FIGURE 2A

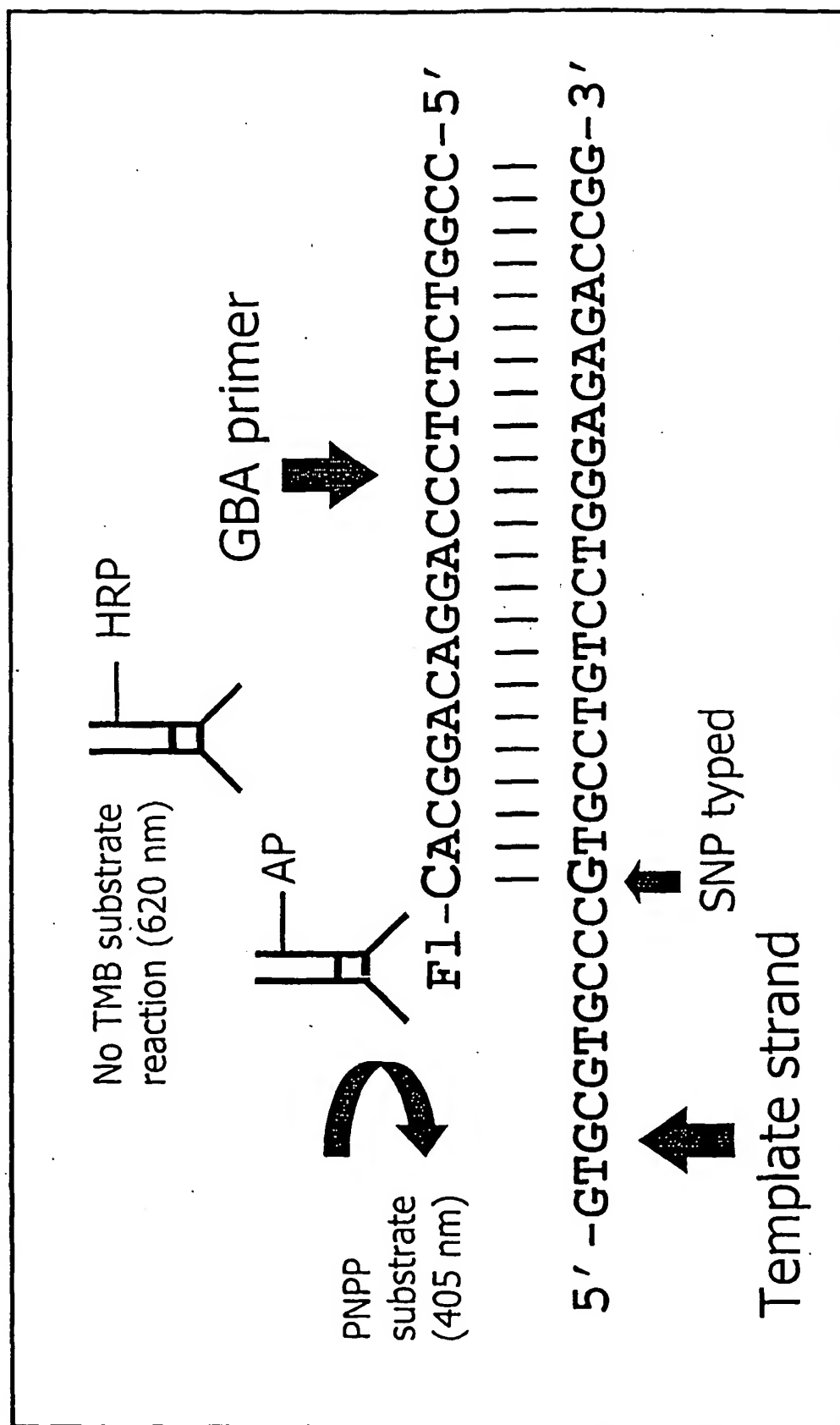


FIGURE 2B

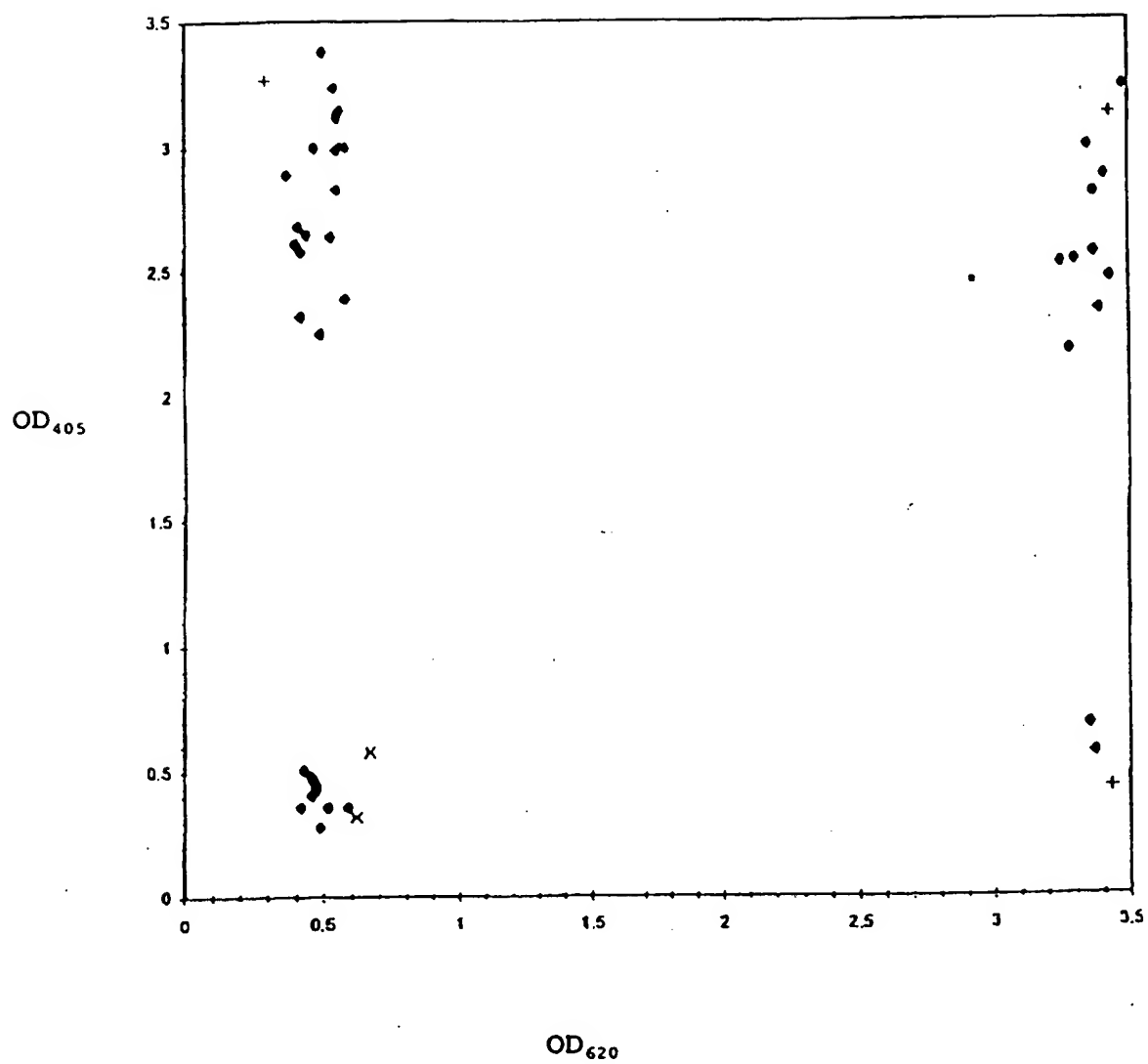


FIGURE 3